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I

Studies of Larval Life and Metamorphosis in Ascidia nigra and Species of Polyandrocarpa

By Caswell Grave and Paul A. Nicoll

Department of Zoology, Washington University, St. Louis

With seventeen tables and seven text figures

[ISSUED October 31, 1939]

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Studies of Larval Life and Metamorphosis in Ascidia nigra and Species of Polyandrocarpa

INTRODUCTION

Experimental studies made during the summer of 1933 (Grave, 1935) demonstrate that a sea-water extract of pharyngeal, atrial, or mantle tissues of adult *Ascidia nigra* is effective in inducing 100 per cent metamorphosis in groups of *Ascidia* larvae within 3 hours after hatching and that a similar extract of tissues of *Polyandrocarpa* induces 100 per cent metamorphosis in groups of *Polyandrocarpa* larvae within 42 minutes after liberation from the parent colony.

These observations led to a search during the summers of 1935 and 1936 for a specific chemical substance in the tissues of these ascidians having the properties required for the rapid acceleration of the process of metamorphosis. Studies were also made to find the time and rate at which metamorphosis occurs in the *Ascidia* larva without artificial acceleration under a standard set of laboratory conditions. An account of the methods and results of this work is given in this paper.

For the opportunities to continue the investigation of ascidian metamorphosis we are indebted to the Carnegie Institution of Washington and especially to the late Dr. William H. Longley, Executive Officer of the Tortugas Laboratory. Dr. Longley's interest in the problem and his eagerness to provide every facility for work toward its solution were always evident.

MATERIALS AND METHODS

The species of larvae with which the studies have been made represent two widely different structural types, one that is liberated in the fully mature state by colonies of *Polyandrocarpa tincta* or *P. gravei,* the other that must be reared from eggs taken from *Ascidia nigra.* Descriptions of these larvae and the methods by which they may be provided for experiment in large numbers have been published (Grave, 1935, pp. 215–218).

Shell vials 20 mm. in diameter and 60 mm. in height, each etched with a horizontal line at the 10-cc. level, have been used as containers of larvae in all experiments with tissue extracts and chemical substances, and the quantity of medium per vial has been uniformly maintained at 10 cc. It was intended also that all vials be stocked with approximately equal numbers of larvae; about 100 if larvae of *Ascidia* were used, about 50 if *Polyandrocarpa.*

In preparation for an experiment all vials were thoroughly washed with fresh water, soap, and brush, and rinsed with fresh water, then with ethyl alcohol. An hour or more before the vials were to be stocked with larvae they were rinsed repeatedly with sea water, then filled with sea water until larvae were available.

The method of stocking vials for an experiment with larvae of *Polyandrocarpa* is as follows: All vials were partially filled with sea water and placed in a planned order on the Mills Water Table until a brood of larvae was in process of liberation. When larvae began to appear at the most illuminated edge of the bowl in which the colony of adult ascidians had been set, they were taken up in a pipette as they accumulated and transferred to vials until each vial was estimated to contain 50 or more. This was continued until all vials were thus stocked or until larvae ceased to be liberated, record being made of the exact time when each vial was stocked. Sea water was added so that all vials contained the same volume. This operation extended over the 15- to 30-minute interval during which the brood was being liberated.

The vials having been stocked, experimental treatment began and was continued during a planned interval or until cases of metamorphosis began to occur at frequent intervals in the vial or group of vials subjected to the method of treatment found to be most effective in inducing metamorphosis. Treatment was then ended by adding formalin to all vials. Counts of the number of larvae were then made and the percentages of metamorphosis calculated, the contents of each vial having been emptied into a watch crystal to facilitate the counting operation.

The method of handling and distributing the smaller *Ascidia* larvae is somewhat different from that just described. Shortly before a batch of *Ascidia* eggs was expected to hatch, all vials to be used in an experiment were arranged in a planned order on the Mills Water Table and a quantity of experimental medium or sea water was placed in each vial just sufficient to amount to 10 cc. when a measured pipette full of larvae was later added. When the most illuminated edge of the water surface of the beakers in which a lot of eggs had developed was swarming with larvae, they were taken up with a pipette and transferred to another beaker, pipette after pipette full, until a sufficient volume to stock the vials had thus been concentrated. Then the larvae, which were kept equally distributed throughout the beaker by agitating the water with a pipette, were transferred to the vials with a special pipette constructed to take up 1 cc. exactly.

The several methods of treatment used during the investigation are described in the sections dealing with experiments and results.

DURATION OF LARVAL LIFE IN ASCIDIA NIGRA

Duration of Larval Life under Standard Laboratory Conditions

Extreme variability in the duration of the free-swimming period of ascidian larvae is characteristic of all known species, extending even to groups of the same age and parentage. This diversity within a species in the length of larval life may be assumed to be primarily due to a corresponding diversity in genetic factors, but the mechanism of metamorphosis is so intimately correlated with the basic organization of the larva that any environmental change may exert a greater or less determining influence upon it.

Throughout this series of studies it has been assumed that the extent to which metamorphosis is accelerated by an experimental stimulus or medium may be satisfactorily measured by the difference between the percentage of metamorphosis occurring in experimentally treated groups of larvae and that taking place in untreated control groups, provided the larvae used in both cases are the same in age and parentage and that all other conditions are identical throughout the period of treatment.

As experience with the larvae of the two species under investigation has increased, however, it has become more and more apparent that the extent of variability in length of larval life is so great as to call for more careful consideration of what constitutes an adequate control of experimental results.

In all critical experiments with larvae of *Ascidia* made since 1930, three control vials have been provided, each stocked with approximately the same number of larvae as those containing groups to be treated, but the wide differences in percentage of metamorphosis that are sometimes found in such a series of control vials, especially when the duration of the experimental treatment is longer than 3 or 4 hours, forces further consideration of the question of the validity of results of experiments. Hence this attempt to ascertain and give some measure of the extent of variability in the duration of larval life in one of the species used in the present study of the process of metamorphosis.

The ease with which large numbers of larvae of *Ascidia nigra* may be obtained at the Tortugas throughout the summer made possible the accumulation of data sufficient for the construction of a series of curves of frequency of metamorphosis of this species under a standardized set of laboratory conditions. These curves are shown in figure 1.

The larvae on which these curves are based were derived in each case from eggs taken from a single large *Ascidia.* As soon as the larvae had swarmed to the surface of the 50-cc. beakers in which the eggs had developed, they were collected with a pipette and concentrated in another similar beaker. From this they were then distributed to a series of shell vials previously arranged on the Mills Water Table in 16 rows of 2, 3, or 4 vials each, all previously filled with sea water to the 9-cc. level. To ensure approximately equal numbers of larvae per vial, their transfer from the stock beaker was made by means of a pipette constructed to take up 1 cc. exactly, and the contents of the stock beaker was agitated before each filling of the transfer pipette. Each vial thus stocked contained a volume of 10 cc. Evaporation was prevented by placing a circular cover glass over the top of each vial. At 3-hour intervals formalin was added to the vials, one row at a time, until 100 per cent metamorphosis had taken place. Counts were then made of the number of larvae and ascidiozooids per vial. The percentages of metamorphosis that occurred in each row of vials were then computed and plotted on a graph as the points determining the position and slope of the curve.

Curve 1 is based upon 3173 larvae that developed June 16 from eggs produced within about one week of the beginning of the spawning season of the species.[1] Owing to an underestimate of the time that would be required for 100 per cent metamorphosis to take place, this curve is incomplete, an insufficient number of vials having been stocked with larvae.

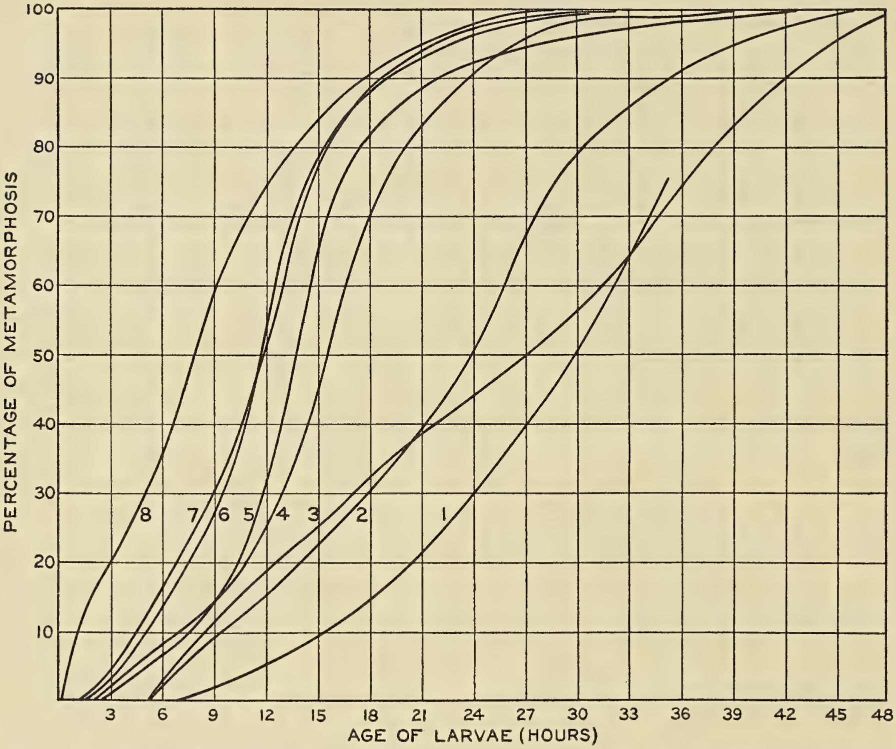

FIG. 1. Curves of frequency of normal metamorphosis of the larva of *Ascidia nigra:*

1. June 16, 1936, based on 3173 larvae.
2. July 5, 1936, based on 2720 larvae.
3. July 5, 1936, based on 2622 larvae.
4. July 7, 1936, based on 2179 larvae.
5. July 7, 1936, based on 3060 larvae.
6. July 18, 1936, based on 5252 larvae.
7. July 23, 1936, based on 4155 larvae.
8. August 1, 1936, based on 3566 larvae.

Eggs taken July 5 from two large *Ascidia* developed into the larvae, 2720 and 2622 respectively, from which curves 2 and 3 are constructed. Curves 4 and 5 are based upon 2179 and 3060 larvae that developed from eggs taken July 7 from two individuals. Curve 6 represents 5252 larvae that developed July 18. Curves 7 and 8 are based on 4155 and 3566 larvae developing from eggs taken July 23 and August 1.

It is apparent from the position and slope of these curves that a seasonal change gradually and progressively occurred in one or more of the factors that determine the duration of the free-swimming period of this larva. Those developing in the earlier part of the spawning season, June 16 and July 5, attained 100 per cent metamorphosis only after an interval of 48 hours, and none metamorphosed until 5 hours after hatching. Larvae produced later in the season, July 23 and August 1, completed 100 per cent metamorphosis in about 30 hours and some of the individuals had a free-swimming period not longer than 1 hour.

---

[1] Seven *Ascidia* examined June 1 were without ripe eggs and two only contained ripe sperm, but on June 8 four of five individuals carried small quantities of ripe eggs. The number that could be taken from a single individual was too small, however, for the requirements of a frequency curve.

The coincidence of this seasonal shift toward a shorter free-swimming period with the rapidly increasing output of eggs by the maturing parent ascidians as the breeding season advanced, suggests that it may be correlated with a gradual decrease in the quantity of yolk substance available for deposition in each egg, and hence may possibly be due to a gradually diminishing amount of energy available in the larva for its maintenance and activity. However, no morphologically observable differences in the yolk content or size of eggs could be noted at different times in the spawning season.

The standard conditions under which the data for these curves were assembled are the same as those under which all recent experiments in the study of acceleration of metamorphosis in *Ascidia* have been made, hence the curves may be considered a universal control from which the validity of all experimental results may be judged. They show that the amount of metamorphosis occurring in experimentally treated groups of larvae which is deductible as possibly due to the inherent tendency to normal metamorphosis, rather than to the experimental agent, becomes greater and greater as the breeding season advances and is always sufficiently large to warn against experiments that require more than 2 or 3 hours for completion. Any experimental agent, such as lactic acid (Grave, 1935), that requires 8 to 14 hours to induce what may be regarded as a significant result as compared with the actual control is to be regarded with suspicion because of the possibility of a high percentage of metamorphosis within such a period due entirely to inherent tendencies.

Duration of Larval Life Influenced by Volume of Water

Observations were made in 1928 showing that the unit volume of sea water per larva has a determining influence on the duration of the free-swimming period of *Ascidia* larvae (Grave, 1935, p. 255–261).

This correlation was made the subject of a series of tests during the summers of 1935 and 1936 to ascertain the extent to which the duration of larval life is affected by differences in the volume of sea water in which larvae are placed soon after hatching. The results of this work all lead to the conclusion that within certain limits, increasing the volume of water per larva increases correspondingly the duration of the free-swimming period of *Ascidia* larvae. A maximum duration of about 5 days is attained by a small percentage of a group of larvae numbering about 450 in a volume of about 250 cc., whereas a like number of larvae of the same parentage and age placed in a volume of 10 cc. reach 100 per cent metamorphosis within 24 hours.

The factor here operating is probably that of the number of mechanical contacts made by a larva in a given unit of time. When a large number of active larvae are crowded together in a small container, collisions of larva with larva and with the side of the container are frequent. These contacts stimulate increased activity, the metabolic by-products of which are essential to acceleration of metamorphosis (Grave, 1935, p. 243).

One of three experiments that demonstrate the correlation between unit volume of sea water per larva and the duration of larval life is presented in outline, as follows:

On June 29, 1936, at 2 P. M., equal numbers of *Ascidia* larvae of the same parentage and age were placed soon after hatching in vessels containing progressively increasing quantities of sea water: a shell vial containing 10 cc. of water plus larvae and a series of pyrex beakers containing 25 cc., 50 cc., 100 cc., 250 cc., 500 cc., and 1000 cc. All containers were partially submerged in the water of the Mills Water Table and covered with glass plates to prevent evaporation. Examinations were made at approximately daily intervals by lifting the containers and observing the larvae in the column of water against the light of a window. Larvae lying on the bottom were made to rise by a quick rotation of the container. All larvae in the 10-cc. vial had metamorphosed before the end of the first 24-hour period. Four larvae only in the 25-cc. beaker were seen to swim at the end of the 48-hour period, all others having metamorphosed, the greater number during the first day as indicated by differences in size of the rapidly growing ascidiozooids. In the beakers of 50 and 100 cc. capacity several larvae swam from the bottom when lifted at the end of the fourth day, and some were present at 5 P. M. July 3, 99 hours after hatching. The following day none were visible in these containers.

In the beakers containing 250, 500, and 1000 cc., no ascidiozooids were seen at the end of the first 24 hours. Swimming larvae in numbers greater than ascidiozooids were present after 72 hours. At 5 P. M. July 3 (99 hours), many motionless larvae could be seen at various levels above the bottom, vertical in position, as if they had reached a perfect density equilibrium with the sea water. All these larvae began to swim within a few moments after the beaker was lifted, feebly at first, then vigorously, as if stimulated by the sudden increase in light intensity. They congregated at the least illuminated part of the beakers and swarmed there with the intense activity characteristic of larvae shortly after hatching, except that they exhibited the *negative* response to light and the *positive* response to gravity, whereas larvae just after hatching show the reverse orientations. At 11:30 A. M. July 5 (141 hours after hatching), a few larvae rose from the bottom of these beakers when exposed to direct sunlight. Numerous ascidiozooids of graded sizes were present at this time, some floating on the surface of the water, some attached to the sides and bottom, showing that metamorphosis had taken place continuously after the first 24 hours. From these results it is not to be inferred that larvae of ascidians in the sea have a long free-swimming life, but that larval life is abnormally prolonged in the motionless water of laboratory containers because of the entire absence of all external stimuli to larval activity. Under laboratory conditions larvae soon cease to swim, and the metabolic by-products of swimming activity that are essential to the activation of the process of metamorphosis are not formed.

Duration of Passage of Eggs in the Oviduct

The occasions when the data for two of the frequency curves of normal metamorphosis of the larva of *Ascidia* were being assembled were taken to ascertain whether duration of the passage of eggs through the oviduct has any influence in determining variability of larvae in any observable character.

Duplicate lots of eggs were collected July 23 from two widely separated segments of the long, tortuous oviduct of a very large *Ascidia* individual, one at the proximal end near the opening into the atrium, the other near the middle of the visceral mass about 5 cm. from the proximal end. Both lots of eggs were fertilized by sperms taken from the same individual and were kept under close observation for any differences that might appear, especially in the time of hatching or in the percentage of each lot which hatched. A large number of the larvae that swarmed to the surface of the beakers were collected and distributed to two sets of vials for determination of the rate and time of metamorphosis. This procedure was repeated on August 1. The data obtained are presented in table 1.

No significant differences in the rate of development, hatching time, swarming time, or duration of larval life of these groups of larvae were observed, hence the two sets of data were combined to form the basis of the curves of frequency 7 and 8, shown in figure 1.

Presumably the large *Ascidia* from which the eggs were taken were in a healthy, thriving condition and their eggs, owing to rapid production, remained in the oviducts for a short time only, certainly not longer than four weeks. There was no opportunity for comparison of these larvae with the progeny of an unhealthy individual whose oviductal fluids might be toxic, hence no light is thrown on the hypothesis of Berrill (1929) that eggs when laid may be physiologically young, normal, or old depending upon influences impressed upon them during passage through the oviduct by the oviductal fluid.

Accelerating Effects of Amino Acids and Related Substances

In planning a program of work for ascertaining the identity of the specific substance in extracts of ascidian tissues that accelerates metamorphosis, the suggestion that it may be one of the amino acids seemed worthy of trial for the reasons that some of these substances have a molecular structure closely similar to that of certain hormones or ferments, and that some have been found to have specific biological effects (Hammett, 1934; Gudernatsch, 1934).

Several amino acids were therefore provided in quantities sufficient for use in preparing media for treatment of groups of larvae.

Sea-water solutions of all these substances were first made in four concentra-

TABLE 1. *Data for curves of frequency of metamorphosis 7 and 8, shown in figure 1*

AGE OF LARVAE WHEN KILLED BY ADDITION OF FORMALIN	LARVAE FROM EGGS IN PROXIMAL SEGMENT OF OVIDUCT				LARVAE FROM EGGS IN MIDDLE SEGMENT OF OVIDUCT			
	1st row of vials		2nd row of vials		1st row of vials		2nd row of vials	
	No. larvae	% metamorphosis	No. larvae	% metamorphosis	No. larvae	% metamorphosis	No. larvae	% metamorphosis
July 23, 1936 (curve 7)								
3 hours............	112	2.6	111	3.6	120	2.5	97	4.1
6 hours............	105	12	121	33	118	15	122	18
9 hours............	111	38	115	28	102	23	119	20
12 hours............	114	66	81	56	84	66	81	44
15 hours............	96	75	105	74	106	86	97	78
18 hours............	114	90	106	70	95	96	122	92
21 hours............	88	84	93	94	102	89	107	93
24 hours............	99	77	97	95	116	90	117	98
27 hours............	83	85	92	94	102	99	114	90
30 hours............	82	70	84	97	117	97	108	93
Average no. larvae per vial...	100	....	101	....	105	....	108	....
Total no. larvae...	1004	....	1015	....	1059	....	1087	....
August 1, 1936 (curve 8)								
3 hours............	67	32	93	15	95	16	114	23
6 hours............	89	36	87	43	101	23	96	41
9 hours............	68	54	60	55	105	66	101	63
12 hours............	77	72	69	73	98	75	98	76
15 hours............	89	77	71	80	106	88	91	87
18 hours............	82	89	76	84	93	77	113	85
21 hours............	77	87	86	94	97	92	89	93
24 hours............	77	96	81	93	100	95	117	91
27 hours............	72	91	88	96	84	90	87	92
30 hours............	80	98	96	100	99	97	97	98
Average no. larvae per vial...	77	....	80	....	97	....	100	....
Total no. larvae...	778	....	807	....	978	....	1003	....

tions. The pH in each case was brought to approximately that of the sea water at the Tortugas by gradually adding small quantities of normal NaOH, tests being made frequently with a glass electrode. When positive results were obtained with the first series of these media, other concentrations were prepared.

The amino acids found to induce a significant percentage of metamorphosis over that occurring in untreated control groups of larvae are listed in the order of their effectiveness: l-histidine, leucine, glycine, cysteine, and d,l-alanine.

Experiments in which groups of larvae of *Ascidia* were placed in these media are outlined in table 2, but the only concentrations included are those yielding the highest percentage of metamorphosis. The table also includes the few tests of amino acids made on groups of *Polyandrocarpa* larvae.

The amino acids that caused little or no acceleration of metamorphosis are: tryptophane, tyrosine, diiodotyrosine, l-aspartic acid, d-glutamic acid, d,l-phenylalanine, d-lysine, and cystine.

TABLE 2. *Experiments with amino acids and ascidian larvae*

Date (1935)	Substance	Concentration	Duration of treatment (hrs.)	No. larvae	Percentage of metamorphosis	
					Treated	Control
		Ascidia nigra				
June 22....	Histidine.....	1:1,000	5	319	82	10.5
July 22.....		1:2,000	3½	135	57	5.0
June 22....	Leucine......	1:2,000	5	262	100	1.0
28....		1:10,000	5	111	30	1.0
29....		1:10,000	3	153	50	2.5
30....		1:4,000	5	145	72	1.0
July 1......		1:4,000	5	139	45	5.0
22....		1:2,000	3½	176	82	5.0
June 24....	Glycine......	1:2,000	5	317	84	9.4
30....		1:4,000	5	153	20	1.0
July 1.....		1:100,000	5	170	24	5.0
22....		1:2,000	3½	135	86	5.0
June 25....	d,l-Alanine....	1:500	5	341	24	2.4
27....		1:100,000	5	77	37	5.0
July 1.....		1:4,000	5	173	25	1.0
June 26....	Cysteine.....	1:1,000	5	206	56	2.3
28....		1:10,000	5	107	11	1.0
29....		1:10,000	3	166	61	2.5
30....		1:20,000	5	149	25	1.0
July 1.....		1:100,000	5	143	83	5.0
		Polyandrocarpa				
July 22....	Histidine.....	1:1,000	6½	24	54	15 (8 hrs.)
		1:1,000	6½	33	93	15 (8 hrs.)
	Leucine......	1:1,000	6½	32	78	15 (8 hrs.)
		1:1,000	6½	26	61	15 (8 hrs.)
	Glycine......	1:1,000	8	36	0	15 (8 hrs.)
		1:1,000	8	50	2	15 (8 hrs.)

At the time these amino acids were being tested it was assumed that the high percentage of metamorphosis induced in certain cases was due to some property of these substances themselves, and this assumption may still be tenable, but, in the light of results of later work with heavy metal salts, it now seems possible that the amino acid preparations that were effective may have contained impurities in the form of heavy metals in sufficient quantities to account for their accelerating potency. Further work with amino acids whose composition has been accurately ascertained will be required before the rôle of amino acids in metamorphosis can be determined.

An Accelerating Substance Extracted from Larvae and Late Embryos

If the substance extracted from tissues of the adult ascidian is the specific agent required for activation of the process of metamorphosis in the larva, it should be present in the larva itself. Several tests were made to find whether the larva of *Ascidia* contains an accelerating substance and whether this is also present in the undeveloped egg and in embryos.

Free-swimming larvae of *Ascidia* in the large quantity necessary for the preparation of the extract were obtained by simultaneously fertilizing batches of eggs taken from several large *Ascidia* individuals. When the resulting larvae were swarming at the surface of the beakers in which they had developed they were transferred to and concentrated in centrifuge tubes. From the semi-solid mass of larvae thus concentrated at the bottom of the tubes all excess water was poured. The larvae were then ground to a pulp in the tubes with a tightly fitting glass rod. This pulp was taken up with a small quantity of sea water and filtered. The combined filtrate, increased to a quantity of 9 cc. by the addition of sea water, was used as a medium for treatment of larvae; 5 cc. was placed in one shell vial of 10 cc. capacity, 3 cc. in another, and 1 cc. in a third, sufficient quantities of sea water and larvae being added to the vials to give final volumes of 10 cc.

The experiments in which an extract of *Ascidia* larvae was tested on larvae of the same species are outlined in table 3. The results demonstrate that larval tissues contain an accelerating substance.

A method slightly different from that by which a pure culture of free-swimming larvae was obtained was required for providing pure cultures of *Ascidia* embryos at particular stages of development. It is as follows: Batches of eggs taken from the oviducts of several large individuals were simultaneously fertilized but kept in separate beakers until segmentation was well advanced. Those batches then found to contain a considerable percentage of infertile or unsegmented eggs were discarded, those only being kept for use in which approximately 100 per cent segmentation occurred; otherwise all extracts intended to be derived from embryos at particular stages would have been diluted and contaminated by that derived from undeveloped eggs.

These several batches of segmenting eggs were poured together into a beaker of 1 liter capacity and thoroughly mixed, then distributed equally to several

100-cc. beakers, in which development was allowed to proceed until the stages were reached of which extracts were desired.

In accordance with this method, sets of extracts were made including eggs at the time of insemination and a series of embryos taken hourly throughout the entire period of development. Three other sets were prepared in which the stages extracted were taken at intervals of 15 minutes throughout that portion only of the period of development during which the accelerating substance was expected to appear, as inferred from results of the previous tests of extracts made at hourly intervals. The method of preparing extracts of eggs and embryos is the same as that used in macerating and extracting free-swimming larvae.

TABLE 3. *Effect of treatment of Ascidia larvae with extract of Ascidia larvae*
(Larvae treated in shell vials each containing 10 cc. of medium)

Date (1935)	Medium	Concentration. (cc. per 10 cc.)	Duration of treatment (hrs.)	Age of larvae	No. larvae	Metamorphosed larvae	
						No.	%
July 4.....	Extract of *Ascidia* larvae	5	4	35 min.	143	102	71
		3	4	35	144	27	18.7
		1	4	35	145	17	11
	Sea water (control)	....	4	35	160	5	3
		....	4	35	128	3	2
		....	4	35	139	12	8.9
July 20....	Extract of *Ascidia* larvae	*	2½	1 hr.	281	85	30
	Sea water (control)	....	2½	1	273	0	0

*The entire lot of extract used in 1 vial, diluted to 10 cc. with sea water.

Owing to unforeseen fluctuations in temperature[2] on certain of the days when the shorter series of extracts were prepared, miscalculations were made as to the time at which stages should be taken covering the period when the accelerating substance is in the process of formation.

The experiments in which these extracts of eggs and embryos were tested on free-swimming larvae of *Ascidia* are brought together in table 4. They show that an accelerating substance is not present in the egg as it comes from the ovary or in embryonic stages of development prior to gastrulation, but that it begins to be formed during late gastrulation when differentiation of tissues takes place.

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[2] The time required for the development of *Ascidia* at the Tortugas, from insemination of eggs to hatching of larvae, varies between 6 hours and 20 minutes and 9 hours, depending upon temperature.

TABLE 4. *Age of the Ascidia embryo at which the accelerating substance is formed*

(Larvae treated in shell vials each containing 10 cc. of extract plus sea water. Number of larvae treated varied between 119 and 148.)

Date	Duration of treatment (hrs.)	Percentage of metamorphosis induced by treatment with extracts of embryos taken at given number of hours after insemination*									Total period of development	Temperature
		0	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.		
July 11, '35......	3½	0	0	0	0	0	5	6	70	......	8 hrs. 15 min.	28.2–28.6° C.
July 12, '35......	2	0	0	0	0	87	97	71	46	100	7 hrs. 40 min.	28.4–29.6° C.
		3½ hrs.	3¾ hrs.	4 hrs.	4¼ hrs.	4½ hrs.	4¾ hrs.	5 hrs.				
July 14, '35......	2½	0	80	81	78	66	82	95			7 hrs. 29 min.	29.2–30.8° C
July 23, '35......	4	0	0	0	77	26	12	49			8 hrs.	26.6–28.2° C
		3 hrs.	3½ hrs.	4 hrs.	4½ hrs.	5 hrs.	5½ hrs.					
July 26, '36......	4	0	27	18	45	55	57				7 hrs. 15 min.	

*Metamorphosis occurred in one control only: 5 per cent in the test made July 26, 1936.

The results also show that prior to the formation of the accelerating substance the eggs and early embryos yield extracts toxic to larvae. Larvae placed in these toxic extracts swim actively for a time, then lie motionless on the bottom of the vial and finally die in a crumpled and distorted condition. The extracts of embryos gradually lose their toxicity as development progresses. The stage immediately preceding gastrulation yields an extract that is non-toxic but without potency in accelerating metamorphosis.

The fluctuations in potency shown by the series of extracts first prepared are probably due to lack of uniformity in maceration of the embryos before extraction. The gradually increasing potency shown by the extracts prepared after the method of maceration had been perfected indicates that the accelerating substance does not appear suddenly in an effective concentration but is formed gradually and continuously as differentiation progresses.

Nature and Properties of the Accelerating Substance

Distribution

The marked acceleration of metamorphosis of ascidian larvae induced by an extract made from desiccated mammalian thyroid gland suggested the use of an extract prepared from dried endostyles of adult ascidians, since endostyle and thyroid are accepted as homologous structures.

A sea-water extract of *Ascidia* endostyle tissue proved to be a most efficient accelerator of metamorphosis of *Ascidia* larvae, but similar extracts prepared from other tissues of *Ascidia* were found to have an equal accelerating effect. It became evident therefore that the substance responsible for the acceleration of metamorphosis has a wide distribution in tissues of the adult ascidian and is not to be identified with a specific secretion of the endostyle. Extracts prepared from several carefully isolated tissues of *Ascidia* and tested simultaneously on groups of *Ascidia* larvae of the same parentage and age showed that all tissues that contain an abundance of muscle fibers yield extracts of about equal accelerating potency. Extracts prepared from tunic, visceral mass, and blood do not induce metamorphosis. The results of experiments with extracts made from different tissues are outlined in table 5.

Species Specificity

In the course of testing the accelerating potency of *Ascidia* tissue extracts on groups of larvae of *Ascidia nigra* and species of *Polyandrocarpa*,[3] a definite

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[3] During the summer of 1933 the species of *Polyandrocarpa* available for work was *P. tincta* exclusively, but in 1935 and especially during 1936, an increasingly greater number of colonies of *P. gravei* were brought to the laboratory. Owing to the great difficulty of distinguishing between these species without a careful comparison of their larvae, some confusion as to the identity of the extracts prepared by the maceration of several colonies was unavoidable. It may be assumed that, in general, larvae of both species respond in a similar manner to treatment with accelerating agents.

TABLE 5. *Diversity in potency of extracts made from various tissues of adult Ascidia nigra*
(Larvae treated in shell vials each containing 10 cc. of medium)

Date	Tissue extracted	Concentration* (cc. per 10 cc.)	Duration of treatment (hrs.)	No. larvae	Percentage of metamorphosis	
					Treated	Control
July 2, '35....	Visceral mass	1.160	3	138	15	0
July 3, '35....	Mantle	1.600	4	94	99	0
	Atrium	0.120	4	61	98	0
July 4, '35....	Atrium	0.630	2½	140	89	8
	Atrium	0.063	2½	169	10	8
July 5, '35....	Blood clot from 3 cc. of blood	?	3	144	1	1
	Blood serum from 3 cc. of blood	?	3	138	12	1
July 8, '35....	Tunic	?	3	?	0	0
July 16, '36....	Visceral mass	?	2	113	5	0

*All concentrations are expressed in cc. of "standard extract" per 10 cc. of medium. The "standard extract" is calculated as if 500 mg. of tissue were extracted with 10 cc. of solvent.

TABLE 6. *Species specificity of ascidian tissue extracts*
(Larvae treated in shell vials each containing 10 cc. of medium)

Date	Tissue extracted	Concentration* (cc. per 10 cc.)	*Ascidia*		*Polyandrocarpa*	
			Duration of treatment (hrs.)	% metamorphosis†	Duration of treatment (hrs.)	% metamorphosis†
Aug. 8, '33..	*Ascidia* mantle-atrium	1.60	2½	100	4	2
	Polyandrocarpa colony	0.19	2½	50	½	100
	Snail, mantle-foot	2.00	2½	5	4	33
	Cynthia mantle	0.54	2½	1	4	0
	Ascidia visceral mass	0.59	2½	4	4	0
Aug. 10, '33..	*Ascidia* mantle-atrium	1.80	2½	100	5	18
	Polyandrocarpa colony	0.13	2½	1	¾	100
	Fish muscle	1.70	2½	7	5	24
	Fish liver	0.85	2½	0	5	11
July 31, '36..	*Polyandrocarpa tincta*	0.62	1¾	22	1½	76‡
	Polyandrocarpa gravei	0.67	1¾	37	1½	96‡
	Ascidia mantle-atrium	0.60	1¾	95	1½	45‡

*Concentrations calculated as for "standard *Ascidia* extract."
†None of the controls for these experiments showed metamorphosis.
‡Larvae of *Polyandrocarpa gravei* used in these experiments, *P. tincta* in all others.

species specificity of the accelerating substance became apparent. The percentage of metamorphosis obtained is much greater when larvae are treated with extracts made from adult tissues of the same species than occurs when they are treated with an extract of a different species. This specificity is quantitative rather than qualitative, since an extract of a foreign species accelerates metamorphosis to a greater extent than occurs in the sea-water controls. *Ascidia* larvae are less specific in their response to tissue extracts than larvae of *Polyandrocarpa*. These specificity relationships are shown in the experiments outlined in table 6.

Standardization of Tissue Extracts

Attempts to establish a basic standard of extract activity were not entirely successful. The changing susceptibility exhibited by larvae to the effect of accelerating agents as the season progressed, and the inherent variations in the potency of extracts prepared under identical conditions from different adult individuals, contributed to this failure. Empirical rules were established, however, for the preparation of extracts of tissues of the two species chiefly involved which permit of an approach to uniformity sufficient for comparisons and verification of experimental results.

In the case of *Ascidia nigra,* the body is removed from the tunic, the heart is punctured to facilitate free drainage of blood, and the mantle, atrium, and pharynx tissues are carefully separated from the remainder of the body. These are then washed in three changes of sea water and gently pressed between filter paper to remove the excess water. Extracts are prepared by grinding 500 mg. of this tissue with 10 cc. of the solvent, the tissue residue being removed by filtration.

In *Polyandrocarpa tincta* and *P. gravei,* the individual zooids are too small to permit the separation of special tissues for extraction. Whole colonies are removed from the objects to which they are attached, freed from débris, dried between sheets of filter paper, ground with the solvent in the ratio of 300 mg. of tissue to 10 cc. of solvent, and filtered.

Extracts so prepared from the tissues of these species are not comparable either in composition or in relative potency, the ratio of mantle-atrium-pharynx tissues per cc. of solvent being much greater in the *Ascidia* preparations than in those derived from the whole colonies of *Polyandrocarpa.* In view of this difference in relative potency, the definite specificity manifested by larvae of *Polyandrocarpa* for extracts of tissues of its own species is even more impressive.

Larvae of *Ascidia* when treated with sea water, or with distilled-water extracts of the parent tissues diluted from 10 to 40 times with sea water, could be expected to undergo 100 per cent metamorphosis within 2 hours. This represents a decrease in the normal duration of larval life of 90 to 95 per cent.

All data of metamorphosis accumulated during two summer seasons from experiments in which groups of *Ascidia* larvae were treated with extracts of *Ascidia* tissues were used for a study of the relation of extract concentration to inductive potency. It was found that an approximately sigmoid curve is obtained by plotting the logarithm of extract concentration against the percentage of metamorphosis induced. This curve is shown in figure 2. The form of the curve is not precise because of certain interfering factors, some of which may be enumerated: high concentrations of extract are toxic to larvae; below the toxicity level a relatively wide range of dilutions have approximately the same accelerating potency; a sharp decrease in induced metamorphosis follows upon continued dilution; the inherent tendency of a certain percentage of larvae to metamorphose without experimental treatment, represented by the cases that occur in controls, tends to give indefiniteness to the end of the curve.

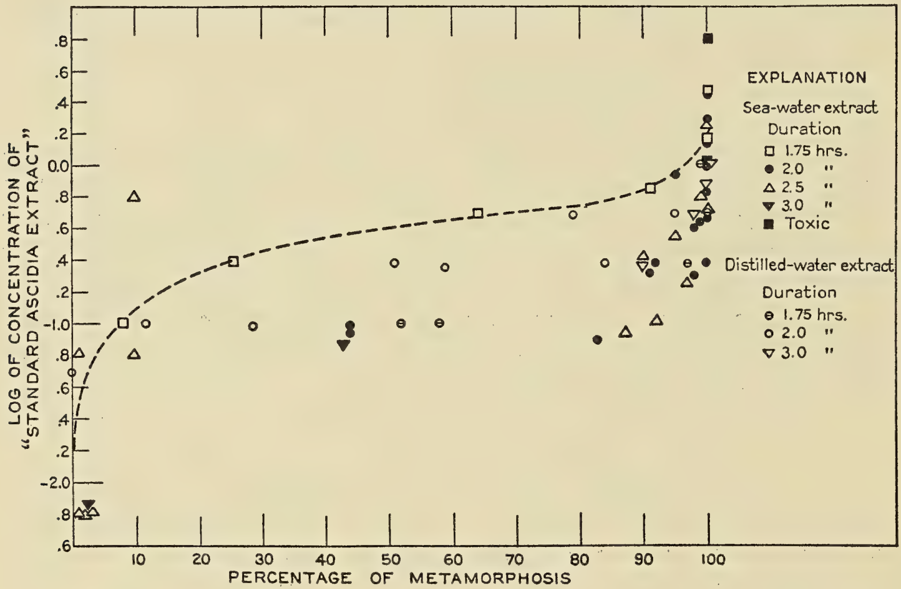

Fig. 2. Induced metamorphosis as a function of extract concentration

The number of points on the curve derived from any given experiment in which conditions were identical are too few for the establishment of an exact relation between concentration of extract and inductive potency, but the combined data indicate a sharp drop in inductive potency below a critical concentration. This appears to be more pronounced with sea-water extracts than with those made with distilled water.

Insolubility in Lipoid Solvents

Dried tissues of *Ascidia,* or evaporated water extracts of the tissues, were extracted with various lipoid solvents and filtered. Both filtrate and residue were then evaporated to dryness at room temperature, extracted with sea water, and again filtered to remove any undissolved material. Experiments, outlined in table 7, in which these extracts were tested for their accelerating potency on groups of *Ascidia* larvae show that the accelerating substance is not taken up by the lipoid solvents and is therefore not lipoidal in nature.

In the first extraction with absolute alcohol, the solvent remained on the tissue for 24 hours and the filtrate when evaporated and taken up with sea water gave an extract with an accelerating potency almost comparable with that prepared from the tissue residue. In the second extraction with alcohol, the tissue was ground with the solvent and immediately filtered. This filtrate yielded an extract that induced a negligible percentage of metamorphosis as compared with that induced by the extract prepared from the tissue residue. It may be assumed that the water taken up from the imperfectly dried tissue by the alcohol, after long extraction, accounts for the accelerating substance in the filtrate, for when the duration of extraction is rapid the amount of water taken up is neglible and the amount of accelerating substance extracted is correspondingly small. The same explanation may be made for the slight accelerating potency of the acetone and benzene filtrates. Chloroform dissolved none of the accelerating substance.

TABLE 7. *Solubilities of accelerating substance in lipoid solvents*
(*Ascidia* larvae treated in shell vials each containing 10 cc. of medium)

Date (1935)	Medium*	Concentration† (cc. per 10 cc.)	Duration of treatment (hrs.)	No. larvae	Percentage of metamorphosis	
					Treated	Control
July 5......	Alcohol extraction:‡					
	Filtrate	2.60	4	139	63	1
	Residue	2.60	4	118	84	1
	Acetone extraction:					
	Filtrate	2.90	4	149	12	1
	Residue	2.90	4	120	100	1
	Sea-water extraction (control)	2.00	4	151	100	1
July 11......	Sea-water extraction (control)	0.19	2½	?	97	0
	Benzene extraction:					
	Filtrate	1.60	2½	139	12	0
	Residue	1.60	2½	127	100	0
	Chloroform extraction:					
	Filtrate	1.30	2½	129	1	0
	Residue	1.30	2½	129	100	0
	Alcohol extraction:§					
	Filtrate	1.60	2½	130	3	0
	Residue	1.60	2½	97	96	0

*The lipoid extracts were evaporated to dryness at room temperature, the residue treated with sea water. This was filtered to remove undissolved material and used in treatment of larvae to determine the amount of accelerating substance extracted by the lipoid solvent.

†Concentrations calculated as "standard *Ascidia* extract."

‡In the first alcohol extraction, the tissue and alcohol after grinding stood 24 hours before filtering.

§The tissue was ground in the alcohol and immediately filtered.

Non-protein in Nature

Distilled-water extracts of *Ascidia* tissues were treated with substances known to precipitate proteins: 2 per cent sulphosalicylic acid and 1 per cent phosphomolybdic acid. The precipitates that appeared were removed and the filtrates brought to the pH of sea water by adding NaOH.

An experiment in which these protein-free extracts were shown to have lost but little of their accelerating potency is outlined in table 8. The slight loss in their potency may be accounted for as due to adsorption of a small amount of the accelerating substance on the protein precipitates.

TABLE 8. *Potency of extracts after elimination of protein substances*
(Larvae treated in shell vials each containing 10 cc. of medium. The pH adjusted to that of fresh sea water.)

Date (1935)	Extract fraction	Concentration* (cc. per 10 cc.)	Duration of treatment (hrs.)	No. larvae	Percentage of metamorphosis	
					Treated	Control
July 9.......	Sea-water extract deproteinized with 2% sulphosalicylic acid	1.58	3½	105	92	6
	Sea-water extract deproteinized with 1% phosphomolybdic acid	1.47	3½	129	98	3

*Concentrations calculated as "standard *Ascidia* extract" per 10 cc. of medium.

Adsorption of the Accelerating Substance

When a distilled-water extract of *Ascidia* tissue was treated with NaOH, raising the pH to approximately 12, a precipitate was formed which was removed by centrifuging. The supernatant fluid was acidified with HCl to the pH of sea water and when tested on groups of larvae was found to have lost a slight amount of the accelerating potency of the original extract. Acidification of the distilled-water extract with HCl and removal of the precipitate that appeared resulted in no decrease in its accelerating potency upon readjustment of the pH. The stability of the accelerating substance toward weak acid and alkali is also shown in other experiments in which the extracts used were prepared by treating the tissue directly with either N/10 HCl or N/10 NaOH. These extracts, after adjustment of the pH to approximately 8.3 in both cases, compared favorably in accelerating potency with the standard water extract. In other cases in which a definite precipitate was formed after the addition of some substance to the distilled-water tissue extract, the resulting solution, after removal of the precipitate, showed some loss in accelerating potency. Thus precipitation of the proteins from extracts with either sulphosalicylic acid or phosphomolybdic acid, previously discussed, reduces to some extent the accelerating potency of the extracts. Since the filtrate obtained after shaking a tissue extract with animal charcoal shows a marked reduction in its accelerating potency, the results in all the cases cited seem to be accounted for as due to partial adsorption of the accelerating substance by the precipitates. Experiments that demonstrate the decrease in accelerating potency of extracts due to adsorption are outlined in table 9.

TABLE 9. *Adsorption of the accelerator substance*

(Larvae of *Ascidia nigra* in 10 cc. of medium in shell vials. The pH adjusted to that of sea water in all tests.)

Date (1935)	Medium	Concentration* (cc. per 10 cc.)	Duration of treatment (hrs.)	No. larvae	Percentage of metamorphosis	
					Treated	Control
July 24....	Distilled-water extract precipitate with NaOH removed, neutralized	0.50	3	175	85	0
	Distilled-water extract	0.25	3	177	100	0
July 26....	N/10 HCl extract neutralized	0.11	5	132	100	9
July 27....	N/10 NaOH extract neutralized	0.14	4	81	92	0
	Distilled-water extract shaken with animal charcoal, filtered	0.50	4	85	3	0

*Concentrations calculated as "standard" extract per 10 cc. of medium.

Heat Stability

The accelerating potency of an extract of *Ascidia* tissue is reduced but slightly by treatment, as follows: refluxing for several hours; evaporating to dryness and dissolving the residue in a volume of water equal to that of the original extract, or drying the tissue in an oven at 180° C. for 2 hours prior to extraction. When the tissue is boiled for several hours in distilled water and the residue is removed by filtration, the filtrate has an accelerating potency nearly equal to that of extracts prepared in the usual way, by grinding the tissue in water at room temperature. On the other hand, slow dehydration of the tissue in a desiccator over H_2SO_4, or prolonged treatment with acetone, or ignition of the tissue in an open crucible, brings about changes which either destroy the accelerating substance or prevent its extraction. Experiments made with tissue extracts that were subjected to various conditions and degrees of heat are outlined in table 10.

TABLE 10. *Heat stability of the accelerating substance*

(Larvae treated in shell vials each containing 10 cc. of medium. *Ascidia* larvae treated with *Ascidia* tissue extracts. *Polyandrocarpa* larvae treated with *Polyandrocarpa* tissue extracts.)

Date (1935)	Extract	Concentration* (cc. per 10 cc.)	Duration of treatment (hrs.)	No. larvae	Percentage of metamorphosis	
					Treated	Control
	Ascidia tissue:					
July 14....	Sea-water extract, evaporated, residue dissolved in distilled water	0.50	1¾	126	93	6
	Sea-water extract of tissue dried at 180° C. 2 hrs.	0.25	1¾	125	92	6
July 15....	Sea-water extract of tissue charred in air	0.30	7¼	159	33	46
July 18....	Sea-water extract of fresh tissue:					
	Refluxed 2 hrs.	0.50	5	157	95	1
	Refluxed 7 hrs.	0.50	5	140	55	1
	Polyandrocarpa tissue:					
July 25....	Distilled-water extract	0.50†	1½	30	90	1
	Refluxed 2 hrs.	1.00	1½	26	100	0
	Refluxed 4 hrs.	1.00	1½	23	100	0

*Concentration calculated as "standard *Ascidia* extract."
†Concentration of *Polyandrocarpa* extracts prepared at ratio of 300 mg. tissue per 10 cc. of solvent.

Dialysis of the Accelerating Substance

Two dialyses were made, in each of which a potent extract was placed inside a small collodion bag and dialyzed 24 hours against an equal volume of distilled water. The external solution in each case was then tested on larvae for its accelerating potency, and small but definitely positive results were obtained. In a third dialysis of 120 hours the external solution had an accelerating potency equal to that of a fresh extract. After 264 hours' dialysis, both external and internal solutions were shown to have approximately the same accelerating potency. Continuous dialysis against distilled water to remove all the accelerating substance did not prove to be practicable under existing conditions at the laboratory. It is probable that all the active material could be removed if dialysis were to be long continued.

Conclusion

These experiments with the accelerating substance limit rather than define its nature. Its insolubility in lipoid solvents, the failure of protein precipitants to remove it from extracts, its stability toward heat short of complete combustion in air, its dialysis across collodion membranes, and its solubility over a wide pH range prove beyond doubt that the accelerating substance is neither lipoid nor protein in nature. These characteristics coupled with the high accelerating potency of extracts at great dilution favor its identification with a simple substance, such as an inorganic salt, and suggest its function to be that of an accelerator or inhibitor of a basic enzymatic reaction controlling initiation of the process of metamorphosis.

The fact that the accelerating substance shows a definite species specificity requires that its molecular structure or its organization with other substances is such as to have been subject to slight changes in the course of evolution of the ascidians.

Accelerating Effects of Inorganic Salts

The results of work with fractions of extracts of adult ascidian tissues described in the preceding section suggested that one or more of the simpler constituents of such extracts may be the substance involved in the acceleration of metamorphosis.

Other facts and observations that supported this suggestion, and led to an investigation of the effect on larvae of low concentrations of copper and iron salts, were: the well-known rôle of heavy metals in extremely low concentrations as accelerators or inhibitors of many biological processes; the remarkable accelerating potency of sea-water extracts made from silica sand known to contain iron as an impurity; the field observation that the most favorable locations at the Tortugas for ascidians are in the vicinity of Fort Jefferson and of wrecked ships, where large amounts of rusting iron are present in the sea water; and the known presence of minute quantities of copper and iron in the tissues of *Ascidia* (Phillips, 1917).

Preliminary experiments with sea-water media containing copper and iron salts gave positive results and the study was therefore extended to include salts of other metals. Tests with iodine solutions were also undertaken to verify the theory advanced by Bradway (1936) that iodine is the agent causing metamorphosis of the larva of *Clavelina.*

Stock solutions of these salts, using Merck's or Mallinckrodt's reagent chemicals, were prepared in accurate concentrations in triple-distilled (all pyrex system) water. The final dilutions with sea water were made immediately before adding larvae to the experimental vials. All concentrations referred to in the following text and tables are expressed in molar values for the final dilution of the added substance without consideration of the concentration of the substance initially present in sea water.

In the preparation of these experimental media the sea water was diluted approximately 10 per cent. This necessitated an investigation of the possible accelerating effect of slightly hypotonic solutions on metamorphosis. For these tests different ratios of sea water to distilled water were prepared by volume. Their effect on larvae was determined in the usual way. The results of two of these experiments, one with *Ascidia,* the other with *Polyandrocarpa* larvae, are given in table 11. They clearly show that no significant accelerating effect is induced when the volume ratio of distilled water to sea water is less than 1 to 5 (approximately 15 per cent distilled water). However, to avoid any possible error from this source, the plan was adopted of diluting all sea-water controls 10 per cent by volume with triple-distilled water. In no experiment was the added salt concentration sufficient to produce hypertonic solutions; that is, the total salt concentration of the medium was never greater than that of unmodified sea water.

TABLE 11. *Effect of hypotonic sea water on metamorphosis*

Date	Distilled water, % of volume	Sea water, % of volume	Duration of treatment (hrs.)	No. larvae	Percentage of metamorphosis	Difference, treated less control
Polyandrocarpa (Volume of medium per vial, 20 cc.)						
July 14, '36...	0	100	3	19	31 (control)	
	10	90	3	15	33	+ 2
	15	85	3	27	55	+24
	20	80	3	19	57	+26
	25	75	3	32	9	−22
	30	70	3	43	toxic	
	40	60	3	31	toxic	
	50	50	3	23	toxic	
*Ascidia** (Volume of medium per vial, 10 cc.)						
Aug. 8, '35....	2	98	2½	209	0	0
	4	96	2½	191	3	3
	6	94	2½	204	2	2
	8	92	2½	164	2	2
	10	90	2½	189	9†	9
	12	88	2½	197	2	2
	14	86	2½	193	5	5
	16	84	2½	211	8	8
	18	82	2½	197	14	14
	20	80	2½	170	13	13

*The sea-water control showed no metamorphosis in 2½ hours.
†The percentage of metamorphosis in this experiment with 10 per cent distilled water is higher than the usual value. Experiments usually showed no greater acceleration than controls.

Copper Salts

Either cupric chloride or nitrate was used with the same results. The small change in anion concentration which, with the chloride ion, was a decrease from its value in sea water due to the 10 per cent dilution, had no effect on metamorphosis. The maximum concentration of the cupric ion possible to obtain in the medium, owing to the solubility of cupric hydroxide at the pH of sea water (8.2 to 8.4 at 28° C.), was found to lie slightly under 1×10^{-4} molar, with respect to added copper. With a concentration of 1×10^{-4} a precipitate appeared within a few minutes after adding the copper salt to sea water, but with 5×10^{-5} molar, or less, the medium remained clear.

Metamorphosis is greatly accelerated in larvae of both types when treated with media containing copper, but the larva of *Polyandrocarpa* responds more readily and to lower concentrations than that of *Ascidia*. The following statements apply, however, to the results of treatment of both: concentrations above 5×10^{-4} molar kill larvae within a few minutes and may be considered toxic to the larval organism as a whole before any effect on the mechanism of metamorphosis occurs. In concentrations below 5×10^{-4} and others as low as 4×10^{-6} molar, the action of copper on the larvae appears to be twofold in that the disruptive phase of metamorphosis with partial retraction of the tail is accelerated but the immediate subsequent growth of the ascidiozooid that occurs in normal metamorphosis does not take place. This toxic effect of copper on growth of the ascidiozooid gradually decreases as the concentration drops, until with values near 4×10^{-6} molar, the young ascidiozooids grow normally provided they are transferred from the copper medium to sea water immediately after the occurrence of the disruptive phase of metamorphosis. The interesting observation was made that under these conditions the young ascidiozooids of both species fail to complete the retraction of the tail and that the protruding portion is eventually lost with the empty larval tunic.

With continued decrease in concentration a narrow range of copper values is found which may be considered optimal in effect on larvae; that is, the disruptive phase of metamorphosis is rapidly induced and normal growth of the young ascidiozooid immediately follows. This range is approximately the same for larvae of both types and lies between 2.5×10^{-6} and 1×10^{-6} molar. Some acceleration of metamorphosis is obtained with even lower concentrations (8×10^{-7} molar), but the onset of the disruptive phase is much longer delayed than with optimal concentrations. When the copper value of the medium was reduced below 8×10^{-7} molar, an actual inhibition of metamorphosis in larvae of both species was obtained as compared with that which occurred in the control vials.

Typical results obtained in experiments in which larvae of *Ascidia* and *Polyandrocarpa* were treated with copper are given in table 12. It should be emphasized that although the optimal concentration of copper is the same for larvae of both types, only *Polyandrocarpa* larvae responded to treatment with copper in a manner comparing favorably with the acceleration of metamorphosis obtained by treatment with extracts of adult ascidian tissue. The time that elapsed before the onset of the disruptive phase in larvae of *Ascidia,* even with copper concentrations slightly toxic to the ascidiozooids, was considerably longer than that required under treatment with tissue extract.

TABLE 12. *Acceleration of metamorphosis by copper salts*

Substance	Concentration (molar)	Duration of treatment (min.)	No. larvae	% metamorphosis	Remarks
			Ascidia		
Sea water	....	120	429	1	Control.
$CuCl_2$	1×10^{-3}	120	118	toxic	Heavy OH precipitation, larvae lived 30 minutes.
	1×10^{-4}	120	129	100	Light OH precipitation, zooids abnormal; no growth.
	5×10^{-5}	100	110	98	No OH precipitation, zooids abnormal; no growth.
	1×10^{-5}	120	147	98	A few zooids grew, very abnormal.
	5×10^{-6}	120	176	72	Zooids grew but tail retraction incomplete.
	2×10^{-6}	120	240	45	Tail retraction complete in a few zooids.
	1×10^{-6}	330	459	80	Growing after 24 hours, many zooids abnormal.
	8×10^{-7}	330	429	34	Growth normal.
	7×10^{-7}	330	417	10	Less metamorphosis than control; inhibition.
	6×10^{-7}	330	459	4	Less metamorphosis than control; inhibition.
Sea water	....	330	540	23	Control.
			Polyandrocarpa		
$CuCl_2$	1×10^{-5}	?	?	toxic	
	2.5×10^{-5}	60	24	100	
Sea water	....	330	21	19	Control.
$CuCl_2$	5×10^{-6}	8	103	100	Zooids growing but abnormal.
	3×10^{-6}	8	54	100	Zooids normal and growing.
	1×10^{-6}	330	61	93	Nearly all zooids normal and growing.
	8×10^{-7}	330	59	71	Nearly all zooids normal and growing.
	6×10^{-7}	330	34	3	Less metamorphosis than control; inhibition.
	4×10^{-7}	330	112	1	Less metamorphosis than control; inhibition.
Sea water	....	270	30	10	Control.
$CuCl_2$	2.5×10^{-6}	45	48	100	Zooids growing; tail retraction incomplete.
	2×10^{-6}	45	35	100	Nearly all zooids complete and growing.
	1.5×10^{-6}	130	25	100	Metamorphosis complete, normal growth.
	1×10^{-6}	240	33	30	Metamorphosis complete, normal growth.
Sea water	....	240	437	2	Control.

The disruptive phase of metamorphosis occurs in larvae of *Polyandrocarpa* between the sixth and the ninth minute after exposure to a concentration of copper slightly above the optimal (4×10^{-6} molar), but the necessary duration of exposure for full activation of the process is much less, approximately 3 minutes. This was determined by exposing groups of larvae to the medium for gradually increasing intervals of time, quickly removing the copper solution after each interval with a large pipette, and adding a like quantity of sea water. In order to make possible the removal of the copper solution from the vials without loss of the larvae, chloretone (0.1 per cent final concentration) was added to the copper medium, which immediately immobilized the larvae without preventing the accelerating effect of the copper. Larvae in the vials from which the copper-chloretone solution was removed after intervals of time too short for metamorphosis to have been induced (1 and 2 minutes) all recovered and swam actively within 30 seconds after the change to sea water. As stated above, the necessary duration of exposure to this copper medium for induction of a high percentage of metamorphosis was thus found to be approximately 3 minutes. Longer exposures before removal of the copper produced toxic effects and the resulting ascidiozooids subsequently showed abnormal growth, while shorter exposures induced metamorphosis in only a small percentage of the larvae. It is of considerable interest, however, that the small number of larvae metamorphosing after the shorter exposures to the copper medium produced ascidiozooids many of which grew and differentiated, while other larvae of the same vials, apparently unaffected by the copper, metamorphosed later but showed gross abnormalities in structure and growth. The abnormal behavior of these larvae may be correlated with the inhibiting effect of low concentrations of copper on the process of metamorphosis, already noted. The results of two series of experiments made to ascertain the time required for copper to activate the process of metamorphosis in larvae of *Polyandrocarpa* are given in table 13.

Iron Salts

Identical results were obtained with the use of ferric chloride or nitrate and ferrous chloride. In contrast to the copper salts, no concentrations of added iron less than 1×10^{-4} molar had any accelerating effect on metamorphosis, and in the higher concentrations, between 1×10^{-4} and 5×10^{-4} molar, by which metamorphosis was accelerated, hydroxide formation with visible precipitation made any interpretation of the data difficult. Concentrations greater than 1×10^{-3} molar were toxic to larvae, but in none of the concentrations of iron in which metamorphosis was induced was there evidence of toxicity to either larvae or ascidiozooids. Removal of the precipitate by filtration yielded solutions which had no accelerating effect on metamorphosis.

The accelerating effect of iron salts is attributed to a specific effect of the ferric or ferrous ion after considerable hesitation, because of the marked acceleration induced by hydroxide precipitates formed in sea water on the addition of NaOH or KOH. Differences in the action of the two solutions suggest that the ac-

TABLE 13. *Duration of copper treatment necessary for metamorphosis of Polyandrocarpa larvae*

($CuCl_2$ concentration 4×10^{-6} molar in all tests. Final concentration chloretone 0.1 per cent in sea water.)

Date (1936)	No. larvae	Duration of Cu treatment (min.)	Metamorphosis		Remarks
			%	Time (min.)	
July 27.........	70	0	2	150	Sea-water control.
	42	0	0	150	Chloretone (0.1 per cent) control.
	25	*	100	10	Copper control (no chloretone).
	51	2	100	19	Larvae recover after change; zooidal growth poor.
	58	3	100	14	Larvae recover after change; zooids normal 24 hours later.
	42	4	100	11	Some larvae recover after change; most zooids abnormal next day.
	37	5	100	9	Some larvae metamorphosed before change; zooids abnormal.
	44	6	100	6	All larvae metamorphosed before change; zooids abnormal.
	?	7	100	....	All larvae metamorphosed before change; zooids abnormal.
	?	8	100	....	All larvae metamorphosed before change; zooids abnormal.
July 29.........	67	0	0	150	Next day, 67 normal zooids (control).
	46	½	0	150	Next day, 43 normal zooids, 3 active larvae.
	77	1	0	150	Next day, 65 normal zooids, 12 active larvae.
	30	1½	3	21	Next day, 23 normal zooids, 7 active larvae.
Aug. 1.........	28	¾	18	15	Next day, 5 normal, 18 abnormal zooids, 5 active larvae.
	43	1	60	14	100 per cent metamorphosis in 60 minutes, many zooids abnormal.
	31	1½	35	13	Next day, 11 normal, 20 abnormal zooids.
	33	2	33	12	Next day, 8 normal, 18 abnormal zooids, 7 active larvae.
	24	2½	100	10	Next day, 17 normal, 7 abnormal zooids.
	83	3	72	9	Next day, 62 normal, 18 abnormal zooids, 2 active larvae.
	43	4	86	8	Next day, 31 normal, 11 abnormal zooids, 1 active larva.

*Cu not removed.

celeration occurring in the iron salts is not due to an effect of the hydroxide precipitate. One important difference is that of the failure of the NaOH treatment to bring about metamorphosis until the precipitate becomes visible as a faint haze in the medium, an epidemic of metamorphosis occurring immediately after the haze appears, while with the iron salt solutions the time course of the accelerating effect is steady and resembles that obtained with copper, aluminum, or tissue extracts, in which hydroxide formation is not involved. Another significant difference is that of the failure of the lowest iron concentration which causes a definite precipitate in the medium (1×10^{-4} molar) to bring about any acceleration of metamorphosis. Failure to induce metamorphosis was also found in the cases of non-toxic concentrations of manganese and cobaltous and ceric chlorides, each of which gave definite precipitates when added to sea water. Finally, the toxic action of slightly higher concentrations of iron (1×10^{-3} molar) on free-swimming larvae is indicative of a specific action of the ferric or ferrous ion on the organism as a whole. In this connection it may also be mentioned that the pH of the NaOH medium is above that of sea water, while the pH of the iron salt media actually falls below that of sea water.

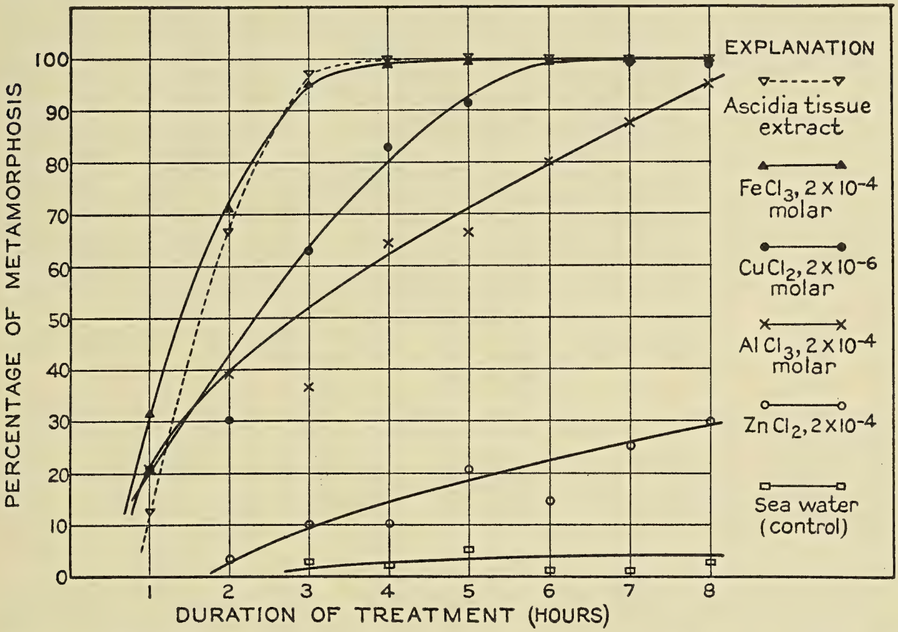

FIG. 3. Acceleration of metamorphosis of *Ascidia* larvae by metallic salts as a function of the duration of treatment. The dotted line shows the acceleration obtained with *Ascidia* tissue extract.

The response of *Ascidia* larvae to iron salts, both in the time of onset of the disruptive phase of metamorphosis and in the subsequent growth of the ascidiozooid, resembles closely their response to extracts of adult ascidian tissue. With larvae of *Polyandrocarpa,* acceleration of metamorphosis by iron salts is somewhat slower than that obtained with optimal concentrations of copper or with

TABLE 14. *Acceleration of metamorphosis by iron salts*

Date	Substance	Concentration (molar)	Duration of treatment (min.)	No. larvae	% metamorphosis	Remarks
			Ascidia			
Aug. 1, '35.....	Sea water	....	120	411	1	Control.
	$FeCl_3$	1 × 10^{-3}	120	137	toxic	Heavy OH precipitation, larvae killed at once.
		5 × 10^{-4}	120	125	95	Heavy OH precipitation, zooids normal, growing.
		2.5 × 10^{-4}	120	143	89	Light OH precipitation, zooids normal, growing.
		1 × 10^{-4}	120	162	2	Hazy OH precipitation, no significant acceleration.
Aug. 2, '35.....	Sea water	....	120	296	1	Control.
	$FeCl_3$	1 × 10^{-3}	100	135	2	Test filtrate free of OH precipitation, no acceleration.
		5 × 10^{-5}	120	102	2	No OH precipitation, no acceleration.
	$FeCl_2$	5 × 10^{-4}	120	144	100	Heavy OH precipitation, tail retraction.
		1 × 10^{-4}	120	141	1	Hazy OH precipitation, no acceleration.
	$Fe(NO_3)_3$	2.5 × 10^{-4}	120	122	71	Light OH precipitation, zooids normal, growing.
		1 × 10^{-4}	120	158	4	Hazy OH precipitation, no acceleration.
			Polyandrocarpa			
Aug. 6, '35.....	Sea water	....	120	41	0	Control.
	$FeCl_3$	5 × 10^{-4}	120	26	58	Heavy OH precipitation, zooids normal.
		2.5 × 10^{-4}	120	26	54	Medium OH precipitation, zooids normal.
		1 × 10^{-4}	120	39	8	Light OH precipitation, zooids normal.
		5 × 10^{-5}	120	42	0	Very light OH precipitation, no acceleration.
July 7, '36.....	Sea water	....	330	27	3	Control.
	$FeCl_3$	3 × 10^{-4}	33	38	100	Medium OH precipitation, zooids normal.
		1 × 10^{-4}	330	32	15	Light OH precipitation, slight acceleration.
		8 × 10^{-4}	330	28	21	No visible OH precipitation, slight acceleration.
	Sea water	....	330	21	19	Control (compare with low iron concentration).

adult tissue extract. If we are to conclude, however, that the accelerative action of ascidian tissue extracts is due to an iron constitutent, the assumption must be made that the iron is present in some combination which keeps it in solution.

Experiments showing the effect of treatment of larvae of both *Ascidia* and *Polyandrocarpa* with iron salts in various concentrations are presented in table 14, and the time course of acceleration of metamorphosis of several substances is shown in figure 3.

Aluminum Salts

A few experiments were made to ascertain the effects of various concentrations of aluminum chloride on metamorphosis. These show that concentrations greater than 2.5×10^{-4} molar are toxic to larvae of both types. With slightly lower concentrations larvae responded with early onset of the disruptive phase and the young ascidiozooids grew normally. No hydroxide precipitate appeared when the salt was added to sea water to form the 2×10^{-4} molar concentration, which therefore may be considered optimal for acceleration of metamorphosis. The time course of the acceleration effect on *Ascidia* larvae of this concentration of aluminum chloride is included in figure 3.

Other Metallic Salts

Three only of several other metallic salts tested for their effect on metamorphosis were found to accelerate the process. Nickel chloride (or nitrate) in a concentration of 2.5×10^{-4} molar had no accelerating effect on larvae of *Polyandrocarpa* and the results of treatment of *Ascidia* larvae were erratic. Zinc chloride in the same concentration caused slight acceleration in *Ascidia* larvae, as shown in figure 3. Calcium chloride had no effect except at the maximum concentration tested (1×10^{-1} molar), and this may have been due to impurities in the calcium salt.

Among the salts found to have no accelerating effect on metamorphosis, vanadium di-chloride is of special interest because the high concentration at which it is present in the blood and tissues of ascidians suggests that it might play a rôle in the process of metamorphosis.

A summary of the results of treatment of *Ascidia* larvae with solutions of all the metallic salts tested is presented in table 15.

Spectographic Analysis of Ascidian Tissues

The strong presumption that one or more of the heavy metals plays an important rôle in the mechanism of ascidian metamorphosis, created by the remarkable accelerating potency of extremely low concentrations of copper and iron salts, requires that heavy metals must be present in some ascidian tissue in quantities adequate for this function, and suggested the importance of a chemical analysis of several of these tissues.

TABLE 15. *Metallic salts as accelerators of metamorphosis of Ascidia larvae*

Date	Substance	Concentration (molar)	Duration of treatment (min.)	Metamorphosis			Remarks
				% of treated	% of control	Acceleration (% of treated less % of control)	
				Accelerators			
June 27, '36...	$CuCl_2$	2×10^{-6}	120	45	1	44	Zooids normal and growing.
Aug. 13, '35...	$Cu(NO_3)_2$	2×10^{-6}	150	36	1	35	Zooids normal and growing.
Aug. 1, '35...	$FeCl_3$	2.5×10^{-4}	120	89	1	88	OH precipitation, zooids normal.
	$Fe(NO_3)_3$	2.5×10^{-4}	120	71	1	70	OH precipitation, zooids normal.
Aug. 14, '35...	$AlCl_3$	2×10^{-4}	120	39	0	39	No OH precipitation, zooids normal.
Aug. 8, '35...	$ZnCl_2$	2.5×10^{-4}	135	15	2	13	No OH precipitation, zooids normal.
	$Ni(NO_3)_2$	2.5×10^{-4}	135	55	2	53	No OH precipitation, activity erratic.
June 27, '36...	$NiCl_2$	5×10^{-4}	120	0	0	0	No OH precipitation, not toxic.
Aug. 7, '35...	$CaCl_2$	1×10^{-1}	205	100	1	99	No OH precipitation, action due to an impurity.
	$CaCl_2$	2×10^{-2}	205	2	1	1	No accelerative action.
				Non-accelerators			
Aug. 7, '35...	$LiNO_3$	1×10^{-2}	205	1	1	0	No OH precipitation, not toxic, no action.
	$MgCl_2$	1×10^{-1}	205	0	1	0	Not toxic (compare with $CaCl_2$).
Aug. 5, '35...	VCl_3	4×10^{-4}	120	3	1	2	Not toxic, no acceleration.
Aug. 8, '35...	$MnCl_2$	1×10^{-2}	135	1	1	0	OH precipitation, no acceleration.
Aug. 10, '35...	$CeCl_3$	5×10^{-4}	205	0	0	0	OH precipitation, no acceleration.
	$CoCl_2$	1×10^{-2}	205	0	0	0	OH precipitation, no acceleration.
	KCl	2×10^{-2}	205	0	0	0	No OH precipitation, not toxic.

Accordingly, during the summer of 1936, *Ascidia* tissue of three varieties (mantle-pharynx-atrium, visceral mass, and whole blood) and whole-colony material of *Polyandrocarpa* were prepared for subsequent analysis, especially for their copper and iron content.

Spectrographic analyses of these samples have now been made through the kindness of Dr. K. B. Thomson, of the Department of Physics of the University of Michigan. They were made on equal weights of the dry tissue and, while not strictly quantitative, give the comparative concentrations of copper and iron present.

The rough approximation of the available heavy metals present in the tissues afforded by this analysis indicates that only copper exists in the order of magnitude necessary to give the concentrations required for acceleration of metamorphosis by the pure metallic salt. The heavy metals in the tissues and tissue extracts, however, may have a synergistic action which would lower the necessary concentration of an individual metal.

No significant difference in the copper and iron content of the three varieties of tissues of *Ascidia* was found, yet the water extracts of these tissues show a very great difference in their accelerating potency. If the potency of the water extract of mantle-pharynx-atrium tissues is due to its copper content, it becomes necessary to account for the lack of accelerating potency of water extracts of visceral mass and whole blood, which contain an equal amount. It is possible that some substance in the visceral mass and blood may prevent extraction of the heavy metals or that some toxic substance is extracted with the heavy metals.

Polyandrocarpa tissue was shown to contain definitely higher concentrations of both copper and iron than were found in tissue of *Ascidia*. Since a much smaller amount of *Polyandrocarpa* tissue is used in the preparation of the standard water extract than is used in the preparation of the standard extract of *Ascidia*, this comparatively greater heavy metal content may account for the apparent equality in potency.

These analyses support but do not prove the hypothesis that extracts of ascidian tissues derive their potency for accelerating the process of metamorphosis from the heavy metals they contain. They afford no indication, however, as to the nature of the combination of the heavy metals in the extracts or tissues.

Iodine

The function of thyroxin in bringing about amphibian metamorphosis has not failed to suggest to many a corresponding mechanism in the metamorphosis of ascidians, although the two processes have little but the name in common. Weiss (1928) and Grave (1935) have shown that sea-water extracts of mammalian whole thyroid gland will accelerate metamorphosis of ascidian larvae, but such an extract contains substances other than thyroxin and its accelerative action affords no proof that thyroxin is the agent of acceleration.

TABLE 16. *Acceleration of metamorphosis by iodine*

Substance	Concentration (molar)	*Ascidia*			*Polyandrocarpa*			Remarks
		Duration of treatment (hrs.)	No. larvae	% metamorphosis	Duration of treatment (min.)	No. larvae	% metamorphosis	
Sea water	....	6	202	4	120	51	0	Control.
	....	20	88	38	120	55	7	Control.
I_2	2×10^{-4}	2	110	100	105	62	95	Some zooids abnormal.
	1×10^{-4}	6	115	16	105	81	40	Not toxic, zooids normal.
	1×10^{-4}	20	106	100	210	41	85	
	5×10^{-5}	6	110	6	120	67	41	
	5×10^{-5}	20	101	100	225	48	90	
	1×10^{-5}	6	109	4	120	27	70	
	1×10^{-5}	20	92	100	210	49	90	
	5×10^{-6}	6	102	6	120	36	66	
	5×10^{-6}	20	86	100	240	46	82	
	1×10^{-6}	6	110	10	120	34	32	
	1×10^{-6}	20	119	100	195	18	22	
	5×10^{-7}	6	104	3	120	21	28	
	5×10^{-7}	20	82	100	240	53	28	
	1×10^{-7}	8	115	0	120	24	0	
	1×10^{-7}	....	....	....	180	16	18	
	5×10^{-8}	8	138	3	....	....	....	
Sea water	....	8	394	8	240	22	31	Control.
KI	1×10^{-3}	6	97	43	120	37	62	I_2 may be freed.
	1×10^{-5}	6	105	18	....	....	....	
KIO_3	1×10^{-2}	2	178	52	105	36	69	
	1×10^{-3}	6	164	55	90	35	86	
NaBr	1×10^{-2}	....	....	....	90	31	9	
	5×10^{-3}	....	....	....	70	63	0	
Sea water	....	....	....	....	100	26	0	Control.

Bradway (1936) found that crystalline thyroxin does not accelerate ascidian metamorphosis, but she regarded the failure as due to the difference between the hormone and crystalline thyroxin. Grave and Nicoll (1936) obtained no acceleration after treatment of ascidian larvae with the related amino acid, diiodotyrosene. Bradway (1936) did find, however, that very dilute solutions of iodine in sea water accelerate metamorphosis in the larva of *Clavelina huntsmani.* Her published data are difficult to interpret with respect to both the nature of the iodine and the concentrations used. In an attempt to repeat her work on larvae of *Ascidia* and *Polyandrocarpa,* her "inorganic iodine" has been assumed to be free iodine and the concentration ratios to be expressed in grams per cubic centimeter.

The treatment of groups of larvae with solutions of iodine both free, as I_2, and combined, as the iodide, KI, or the iodate, KIO_3, are presented in table 16. The accelerating effect of iodine is verified, although the concentrations required were much greater than those found by Bradway to accelerate metamorphosis in the larva of *Clavelina.* Concentrations less than 2×10^{-4} molar I_2 were not toxic to larvae of either *Ascidia* or *Polyandrocarpa,* whereas Bradway reports toxic effects with concentrations as low as 1×10^{-7} molar (her value is expressed as 1 : 40,000,000). The acceleration of metamorphosis following treatment of larvae with iodine solutions is much slower than that obtained by treatment with heavy metal salts or tissue extracts, or even with sea-water extracts of mammalian thyroid gland (Grave, 1935). It is probably not to be identified in any direct way with the naturally occurring process of metamorphosis, but rather is to be interpreted as a response of the larva to a foreign substance. The accelerative action of the iodide and iodate, which occurs only at relatively high concentrations, may be due to reduction of the salts with the liberation of free iodine.

Diversities in Physical and Chemical Induction

A brief statement is here given of the problem whether, in the light of marked differences in structural organization and physiological responses between the types of larvae represented by species of *Polyandrocarpa* and *Ascidia,* it is possible to conclude that the mechanism of metamorphosis is fundamentally the same in both and hence that this mechanism is common to all ascidians.

The Polyandrocarpa Larva

Physically induced and normal metamorphosis of the larva of *Polyandrocarpa* do not take place until two conditioning processes have been completed, one depending upon aging or differentiation of the larva, the other resulting from an accumulation of metabolic by-products of swimming activity. The time interval required for the first of these conditioning processes to appear varies

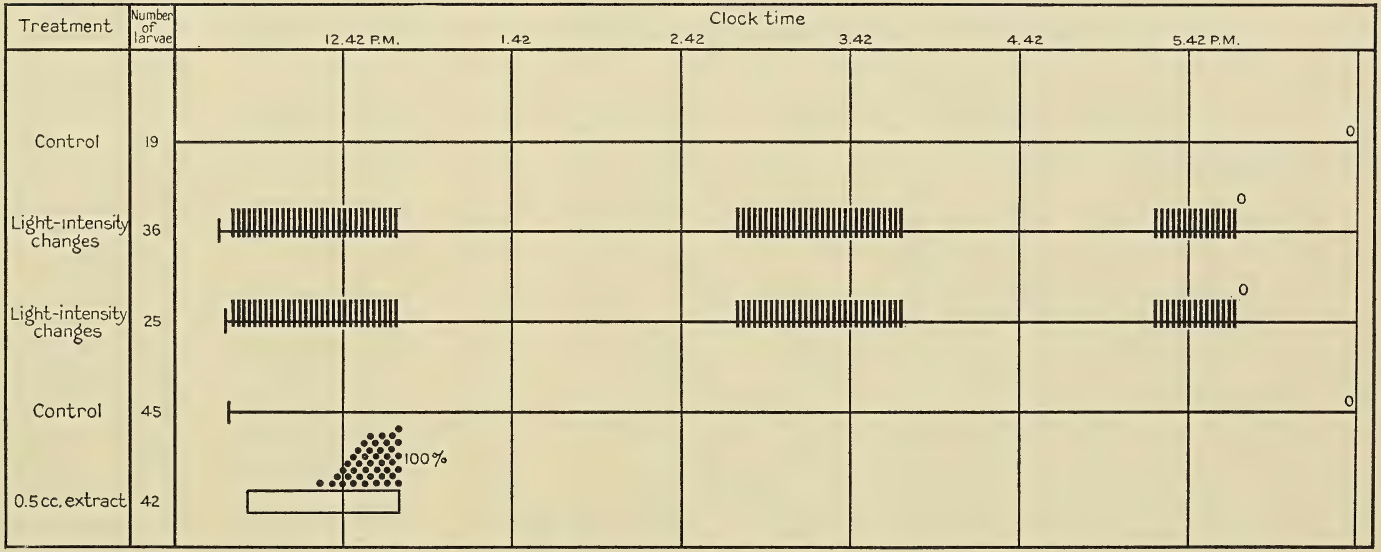

FIG. 4. Experiment 229. July 25, 1935. Demonstrating that an aging factor, which is essential for induction of metamorphosis of the *Polyandrocarpa* larva by physical stimulation, is not essential for induction of metamorphosis by an extract of *Polyandrocarpa* tissues. The series of black bars represent stimulations of groups of larvae by repeated changes in light intensity. Each black circle represents the time of metamorphosis of a larva. Other explanations in the text.

greatly in different broods of larvae: 40 minutes to several hours.[4] After the aging factor has appeared, a short period only of swimming activity is required for the activation of the process of metamorphosis.

Chemical induction of metamorphosis, on the other hand, can be quickly brought about in larvae of all broods in the entire absence of either of these conditioning factors.

This difference between physical and chemical induction in the *Polyandrocarpa* larva is demonstrated by three experiments outlined in figures 4, 5, and 6. In each experiment groups of larvae of the same brood were simultaneously treated by physical and chemical methods of accelerating metamorphosis. In figure 4, experiment 229, the aging factor had not appeared at the end of 6 hours after liberation of the larvae, hence the three series of physical stimulations by sudden changes in light intensity[5] to which the groups of larvae in two vials were subjected failed to induce metamorphosis, while the group of 42 larvae of the same brood that was placed in tissue extract all metamorphosed in less than an hour. In figure 5, experiment 234, the aging factor developed during the third and fourth hours after liberation, hence the first series of 92 light-intensity changes, delivered during the first and second hours, had no effect, while the second series of 50 stimulations, made during the fourth hour, induced 40 per cent and 16 per cent metamorphosis respectively in the two groups of larvae so treated. All larvae of the group treated with tissue extract metamorphosed within 80 minutes. In figure 6, experiment 235, the first series of 60 light-intensity changes was delivered before the aging factor had developed and had no accelerating effect, but the second series of 50 stimulations given during the period of aging caused metamorphosis of 64 per cent of the group. Eighty-one larvae of the same brood placed in tissue extract all metamorphosed within 54 minutes.

There is no waiting for either the aging process or the accumulation of by-products of swimming metabolism when larvae of *Polyandrocarpa* are placed

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[4] It is possible that the time of liberation of broods of larvae by colonies of *Polyandrocarpa* has no constant relation to the time of hatching of the larvae. Some broods may be retained in the brood chambers until many of the larvae are far advanced in the aging process, while others may be liberated so soon after the larvae have hatched that several hours must elapse before the aging factor appears. The time interval between hatching and development of the aging factor may therefore be as constant in *Polyandrocarpa* as it has been shown to be in *Ascidia*.

[5] The most effective physical method of accelerating metamorphosis of conditioned larvae of the *Polyandrocarpa* type consists in alternately covering and uncovering vials containing larvae with a tumbler jacketed with black paper, at 1-minute intervals, thus suddenly reducing and increasing the intensity of light. The efficacy of this method is due to the fact that all larvae are stimulated by each change in light intensity to immediate and vigorous swimming activity, hence the rapid production and accumulation of metabolic by-products of neuro-muscular activity as the treatment is continued. An epidemic of metamorphosis in a group of larvae that has acquired the aging factor may be expected to begin at about the twentieth change in light intensity and to involve all larvae so conditioned. In the figures, each series of such physical treatment is represented by an alternate series of black and white bars drawn to the clock-time scale.

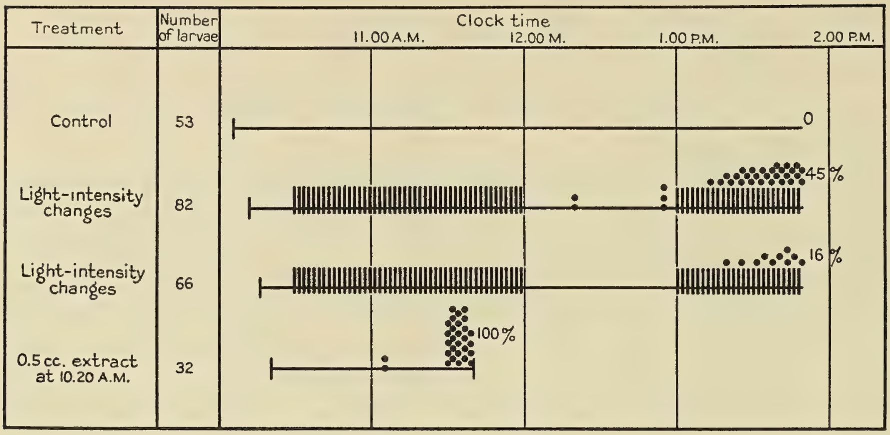

Fig. 5. Experiment 234. July 28, 1935. Demonstrating that metamorphosis cannot be induced in the larva of *Polyandrocarpa* by physical stimulation until a factor due to aging of the larva is present, but that metamorphosis is quickly induced by an extract of *Polyandrocarpa* tissues in complete independence of the aging factor. The series of black bars represent stimulation of groups of larvae by repeated changes in light intensity. Each black circle represents the time of metamorphosis of a larva. Other explanations in the text.

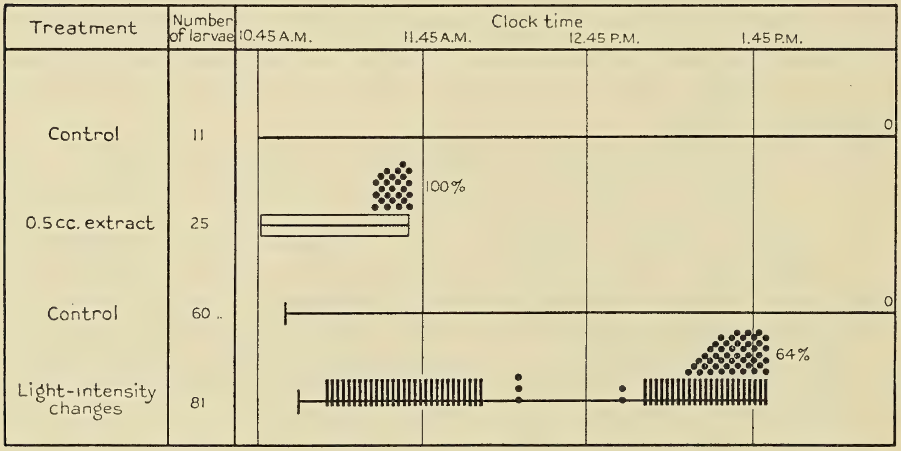

Fig. 6. Experiment 235. July 28, 1935. Demonstrating that metamorphosis can be induced in the larva of *Polyandrocarpa* by physical stimulation only after an aging factor has been developed, but that induction of metamorphosis by treatment with an extract of *Polyandrocarpa* tissues is entirely independent of the aging factor. The series of black bars represent stimulation of groups of larvae by sudden repeated changes in light intensity. Each black circle represents the time of metamorphosis of a larva. Other explanations in the text.

in a water extract of tissue of adult *Polyandrocarpa.* Metamorphosis occurs quickly in larvae of all broods in apparent complete independence of both conditioning factors. This independence of the effects of aging and swimming activity is even more marked when groups of these larvae are placed in low concentrations of copper and iron salts. The disruptive process occurs almost immediately (see tables 12, 13, and 14). This difference between physical and chemical induction in *Polyandrocarpa* may be accounted for if it is assumed that tissue extracts and other chemical media that are potent in the rapid acceleration of metamorphosis contain the specific substance required for catalyzing the disruptive process, hence the larva is freed from the necessity of synthesizing the specific accelerating substance from chemical by-products of the aging process and its own neuro-muscular activity.

The Larva of Ascidia nigra

In the process of metamorphosis of the type of larva found in *Ascidia nigra,* it is possible to infer the operation of the same conditioning factors or physiological states that appear to be involved in normal metamorphosis of the larva of *Polyandrocarpa,* but the inferences do not rest upon so firm a basis of observation.

The presence of a "susceptibility" factor due to an aging process after hatching would have been especially difficult to recognize in the larva of *Ascidia* were it not for the background of plainer evidence of its existence in the *Polyandrocarpa* larva. In all batches of larvae of *Ascidia* the aging factor has been found to make its first appearance in a relatively constant time after hatching, but it varies with the time in the spawning season from about 5 hours to 1 hour. The presence of the aging factor is first shown by a small percentage of the larvae of each batch. It then continues to develop at about the same percentage rate throughout the entire free-swimming period of about 30 to 48 hours.

Proof that an aging factor is involved in preparing the *Ascidia* larva for metamorphosis is afforded by the results of several experiments made in 1935, one of which is outlined in figure 7. In this experiment larvae that began to hatch at 8:40 A. M. on July 21, from eggs fertilized at 12:10 midnight, were collected and distributed to 10 shell vials of 10 cc. capacity at 9:20 A. M. At successive hourly intervals, beginning with the first vial at 9:20 A. M., 0.5 cc. of standard extract of *Ascidia nigra* tissue was added to the vials. Close watch was kept to see when the first larva metamorphosed in each vial after adding the tissue extract and to note when metamorphosis reached 100 per cent. The results show that 2 hours and 10 minutes were required for the induction of 97 per cent metamorphosis in the group of larvae in the first vial, which was placed in the extract 35 minutes after hatching, and that the time intervals required for the induction of approximately 100 per cent metamorphosis in the other vials gradually shortened as the larvae aged until, at the end of the seventh hour, an interval of 22 minutes only was required after adding the extract. Normal metamorphosis began to occur in these groups of larvae during the fourth hour after hatching, 3 zooids being observed in vial 5 at 1:20 P. M.; 5 zooids in vial 6 at 2:20; 5 zooids in vial 7 at 3:20; 8 zooids in vial 8 at 4:20; and 5 zooids in vial 9 at 5:20 P. M.

Evidence that metamorphosis occurs in *Ascidia* larvae very soon after the development of the aging factor may be inferred from the data forming the basis for the several frequency curves shown in figure 1. This larva seems to be able to retain and to concentrate the by-products of its swimming activities and is thus in a condition to react with the aging factor as soon as it appears, whereas, in the larva of *Polyandrocarpa,* the by-products of neuro-muscular metabolism seem to diffuse rapidly and to be lost to the sea water, thus necessitating renewed swimming activity after the appearance of the aging factor.

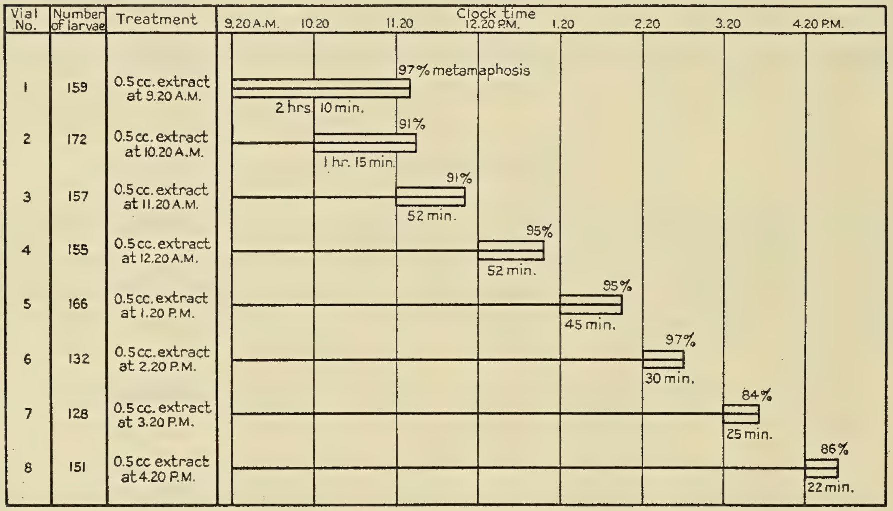

FIG. 7. Experiment 225. July 21, 1935. Demonstrating that induction of metamorphosis in the larva of *Ascidia nigra* by an extract of the tissue of the adult ascidian is conditioned by a factor that develops in the larva with aging. Explanations in the text.

That swimming activity plays the same rôle in the induction of normal metamorphosis in the larva of *Ascidia* as in that of *Polyandrocarpa,* ample evidence is afforded by experiments made in 1930 in which the long-continued swimming activity of groups of these larvae, stimulated by the light-intensity changes that automatically occurred in vials banded at the bottom with black paper, induced high percentages of metamorphosis far in advance of its occurrence in the inactive larvae in control vials (Grave, 1935, pp. 236–239).

The larva of *Ascidia* differs from that of *Polyandrocarpa* in that the conditioning factors due to aging and swimming activity seem to play a rôle as necessary in the induction of metamorphosis by tissue extracts and metal salts as in the physically induced process. The independence of these factors shown by the *Polyandrocarpa* larva when placed in tissue extracts and metal salts is not shown by the larva of *Ascidia nigra.*

THEORETICAL INTERPRETATIONS

On the basis of earlier studies (Grave, 1935) of normal metamorphosis in larvae of *Polyandrocarpa,* it was inferred that the process is conditioned by two factors, one that appears with an "aging" of the larva after its liberation from the parent colony, another dependent upon its swimming activities. Since metamorphosis was never observed to occur when one of these conditioning factors was present alone, but only when both occurred together, it was concluded that both factors are chemical in nature and that these chemical substances probably interact to form a third substance having the properties required to activate or catalyze the disruptive process of metamorphosis. The first factor was therefore assumed to be a substance formed or secreted by larval organs or tissues after they have reached the necessary stage of differentiation; the second was assumed to be one of the metabolic by-products resulting from swimming activities of the larva.

With the knowledge that has accumulated in the course of later studies of the diverse conditions under which metamorphosis can be induced, it becomes plain that the processes involved are far too complex to be included in any simple chemical reaction.

The earlier experiments dealing only with physical methods and agents do demonstrate the importance of changes in the larva that come with age and with its own swimming activity for the determination of the length of larval life, but they offer insight neither into the method of the control of its duration nor into the nature of the underlying mechanism of metamorphosis.

The problem of the mechanism of metamorphosis in ascidians must be approached in the light of conditions peculiar to this group of animals. The larva is a dual organism whose larval action system not only is independent of the action system of the ascidiozooid, but completely inhibits growth of the latter. The larval organs and structures are not transformed into those of the adult except as their degenerating remains provide nutrient material for growth and differentiation of the ascidiozooid. The larva takes no food during the free-swimming period, but is wholly dependent for its energy requirements upon the yolk stored in the egg. It is easily possible to obtain normal and rapidly growing ascidiozooids from the body portion of the larva by severing the tail soon after hatching. Metamorphosis of these non-motile, tailless bodies is accelerated by the same chemical agents that induce the process in active whole larvae.

It is of interest to note in this connection that the severed tails remain active for several hours, even exhibiting the same or similar bursts of swimming movement observed in the larva.

Metamorphosis in ascidians appears therefore to be a sudden breakdown of the larval action system immediately followed by the resumption of growth and differentiation of the action system of the ascidiozooid. Evidence of a causal relationship in either direction between these two processes is somewhat conflicting, but, in general, the disruption of the larval action system appears to release the ascidiozooid from an inhibitory influence. A few cases are known in which the action system of the ascidiozooid proceeded with its growth and differentiation processes without disruption of the larva, but they belong to the life cycle of species (*Ecteinascidia, Perophora*) in which the structures pertaining to the adult form are much further differentiated in the larva than in the species dealt with in this paper, hence inhibition is not so pronounced. Death is the usual fate of a larva that fails to metamorphose. Other evidence indicating that no necessary relationship exists between these two action systems is afforded by the series of experiments in which larvae were treated with high concentrations of copper salts (4×10^{-6} molar). Under these conditions the larval action system undergoes the disruptive phase of metamorphosis within 7 minutes, but only after several hours does the ascidiozooid show the degree of growth that is normally observed within an hour. This interval of retardation between the disruptive process and growth is not eliminated by the immediate removal of the copper, i. e., by transfer of the metamorphosing larvae to fresh sea water.

Since many wholly unrelated and different environmental changes are effective in inducing metamorphosis, it is apparent that no necessary relationship exists between the disruptive process and the stimulus by which it is initiated, although it does not follow that the final condition preceding the disruptive process may not in each case be the same. It is possible therefore that interference with or prevention of the continuation of larval life, however effected, may be the cause of metamorphosis.

The dependence of the larva upon its initial store of yolk for all its metabolic processes during larval life suggests this store of available energy as the factor that may be involved in determining the duration of larval life. The marked structural diversity between larva and adult ascidian suggests the possibility that an even more basic difference may distinguish them. A higher rate of metabolism in the larval action system than that in the ascidiozooid may be responsible for the apparent domination of the larva over the ascidiozooid and for the inhibition of growth and differentiation of the latter. Many such gradients have been described in the physiology of cells, tissues, and organ systems. If such a difference exists in the metabolism of larva and ascidiozooid, we might expect to find enzyme systems necessary for larval life not required for the metabolic processes of adult life.

Consideration of the dependence of the larva on food stores in the egg suggests the presence of a highly efficient system for resynthesis of the necessary substrates. In such a system specific enzymes must play a dominant rôle. Any material therefore that poisons these enzymes, either wholly or in part, would decrease the length of larval life and hasten the appearance of metamorphosis. The concentration of the poison, with its dependent degree of inactivation of the enzyme and reduction of the total amount of activity, or of the amount of activity per unit of time, would be an important factor in the control of the duration of larval life.

The rate of action of such an enzyme poison would be influenced by several factors, some of the more important of which are suggested: the concentration of the poison in the surrounding media; the degree of permeability of the larval tunic or mantle; diffusion time for distribution throughout the larva; and conditions within the larva with regard to concentration levels of enzyme or substrate.

In the absence of specific proof that a mechanism of the kind outlined actually conditions ascidian metamorphosis, it may be pointed out that the most active of the accelerating substances discovered, namely copper salts, are known to inhibit several enzyme systems, especially those containing sulphydryl groups. Tissue extracts may depend upon their content of copper, iron, or other metallic salts for their accelerating potency. The colloidal hydroxides which induce early metamorphosis probably exert their effect on the larva in another manner, but the possibility remains that their action may be due to the production of marked changes in the permeability of the hemicellulose substance of the larval tunic.

The relation between concentration and percentage of acceleration of metamorphosis has been clearly evident in all experiments in which metallic salts were the accelerating agents. A somewhat similar relationship is found to hold in the case of tissue extracts, but a different controlling factor must be involved in the observed decrease in the time necessary for a given concentration of extract to induce early metamorphosis as the age of larvae increases. The data presented in the first part of table 17 clearly show this time decrease despite the fact that the larvae were kept inactive by low concentrations of chloretone from the time of hatching until the tissue extract was added to the vials. During the first 6 hours the time required for the acceleration medium to induce a high percentage of metamorphosis is steadily reduced, then a more or less limiting value is reached and maintained (fig. 7). Larvae 5 days old were shown by other experiments to require this minimal time for induction of metamorphosis by tissue extract. The fact that this decrease in the induction time of larvae with aging is independent of swimming activity points to a permeability change in the tunic or mantle as the controlling factor. The conclusion that swimming activity of larvae plays a secondary rôle in the induction of early metamorphosis by accelerating agents of all types is supported by the results of experiments outlined in the second part of table 17. Here the percentages of metamorphosis that occur after approximately the same time intervals show the action of a given concentration of the accelerating medium with and without the use of chloretone. In some instances the time interval of treatment with chloretone plus medium was lengthened in order to demonstrate that metamorphosis may be induced in entire absence of swimming activity. The influence of larval swimming activity in early induction of metamorphosis, as might be expected, is more marked when just-subthreshold concentrations of the accelerating medium are used, the term "subthreshold" being used to denote a concentration of the medium that does not immediately produce conditions incompatible with continued life of the larvae.

These inferences and interpretations concerning the nature of the mechanism

TABLE 17. *Inhibition of normal and induced metamorphosis by chloretone*

Date	Medium	Concentration	Duration of treatment (min.)	No. larvae	% metamorphosis	Remarks
		Ascidia				
July 18, '35.....	Sea water	....	1140	106	100	Control.
	Sea water + chloretone	0.05%	1140	125	6	
July 21, '35.....	Sea water	....	502	167	19	Control.
	Sea water + chloretone	0.05%	510	133	0.03	
July 18, '35.....	Tissue extract	0.5 cc. (standard)	150	136	95	
	Tissue extract + chloretone*	0.5 cc. + 0.05%	300	100	0	
	Tissue extract + chloretone	0.5 cc. + 0.05%	360	143	55	
July 21, '35.....	Tissue extract	0.5 cc. (standard)	130	159	97	
	Tissue extract + chloretone	0.5 cc. + 0.05%	180	155	0	
		Polyandrocarpa				
July 31, '35.....	Sea water	....	286	29	14	Control.
	Sea water + chloretone	0.05%	290	29	3	
July 25, '35.....	NaOH + sea water	0.175 cc. N to 10 cc.	113	33	100	OH precipitation appeared 56 minutes after NaOH added.
	NaOH + sea water + chloretone	0.175 cc. + 0.05%	155	37	73	OH precipitation appeared 65 minutes after NaOH added.
July 19, '36.....	Tissue extract	2 cc. to 10 cc.	330	48	83	
	Tissue extract + chloretone	2 cc. + 0.05%	330	86	1	Although the acceleration due to extract is very slow, chloretone inhibits this acceleration.
July 19, '36.....	$CuCl_2$	4×10^{-6} M	7	81	100	Growth of zooids abnormal.
	$CuCl_2$ + chloretone	4×10^{-6} M + 0.05%	9	59	100	Growth of zooids abnormal.
July 18, '36.....	$CuCl_2$	2×10^{-6} M	15	32	100	Many normal zooids.
	$CuCl_2$ + chloretone	2×10^{-6} M + 0.05%	31	24	100	Many normal zooids.
	$CuCl_2$	1×10^{-6} M	76	34	91	Many normal zooids.
	$CuCl_2$ + chloretone	1×10^{-6} M + 0.05%	76	48	2	Many normal zooids.
	$CuCl_2$	8×10^{-7} M	65	89	14	Many normal zooids.
	$CuCl_2$ + chloretone	8×10^{-7} M + 0.05%	65	94	4	Many normal zooids.

*The tissue extract plus chloretone removed after 300 minutes and replaced with sea water. 100 per cent metamorphosis occurred in 50 minutes.

of ascidian metamorphosis and the rôle of certain substances in accelerating the process rest upon a foundation of fact and observation far from complete, and are to be regarded as hypothetical and tentative. Further study of the problem will doubtless lead to modifications of views that seem to harmonize with facts now known.

Summary of Results

1. The amino acids l-histidine, leucine, glycine, cysteine, and d,l-alanine, in the form received from the laboratories in which they were prepared, accelerated metamorphosis in groups of larvae of both types.
2. The amino acids tryptophane, tyrosine, diiodotyrosine, l-aspartic acid, d-glutamic acid, d,l-phenylalanine, d-lysine, and cystine did not induce metamorphosis in groups of larvae of either type.
3. A sea-water extract of free-swimming larvae or of late embryonic stages of *Ascidia* has the same accelerating effect on metamorphosis of *Ascidia* larvae as similar extracts made from tissues of the adult ascidian.
4. Sea-water extracts of ovarian eggs or of early embryos of *Ascidia* are toxic to *Ascidia* larvae and do not induce metamorphosis.
5. Distilled-water extracts of *Ascidia* tissue lose little or none of their potency to induce early metamorphosis after deproteinization by the common protein precipitants. The protein fractions do not accelerate metamorphosis.
6. Extraction of adult *Ascidia* tissue or distilled-water extracts of the tissue with lipoid solvents such as ethyl alcohol, acetone, benzene, or chloroform does not remove the accelerating substance.
7. Heating adult *Ascidia* tissue or refluxing distilled-water extracts of the tissue for several hours does not destroy the accelerating substance.
8. The accelerating substance present in distilled-water extracts of *Ascidia* tissue dialyzes through a collodion membrane prepared from stock collodion.
9. Non-toxic concentrations of copper, iron, and aluminum salts induce early metamorphosis to a marked degree. Slight acceleration only is obtained with higher concentrations of zinc, nickel, and calcium salts.
10. Non-toxic concentrations of $LiNO_3$, $MgCl_2$, VCl_2, $MnCl_2$, $CeCl_3$, $CoCl_2$, and KCl have no accelerating effect on metamorphosis.
11. Iodine, as I_2, in a molar concentration of 2×10^{-4}, induced 100 per cent metamorphosis in *Ascidia* larvae after 2 hours treatment, and 95 per cent metamorphosis in groups of *Polyandrocarpa* larvae after 105 minutes.
12. The duration of the free-swimming period of *Ascidia* larvae is longest at the beginning of the breeding season of the species and becomes gradually shorter as the season advances.
13. Changes in the unit volume of sea water per larva greatly affect the duration of the free-swimming period of *Ascidia* larvae: About 450 larvae in a shell vial of 10 cc. capacity undergo 100 per cent metamorphosis in 24 hours, but if the volume of water is increased to 250 cc., about 5 days are required for metamorphosis of the entire group.

14. Chloretone (0.5 cc. of a 1 per cent solution in sea water, in a volume of 10 cc.) greatly inhibits but does not entirely stop the process of metamorphosis. It also inhibits but does not entirely prevent the accelerating action of tissue extracts and the heavy metal salts.

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PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

II

Ecteinascidia tortugensis, Species Nova With a Review of the Perophoridae (Ascidiacea) of the Tortugas

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With five color plates and three text figures

[ISSUED October 31, 1939]

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Ecteinascidia tortugensis, Species Nova With a Review of the Perophoridae (Ascidiacea) of the Tortugas

INTRODUCTION

During the season of 1936 at the Tortugas Laboratory the senior investigator undertook a study of the regeneration of pieces cut from the growing stolons of several species of the family Perophoridae (Ascidiacea Phlebobranchia) which grow in profusion at many places in the Tortugas area. It was discovered that portions of stolons from different species could be fused together and would eventually form composite colonies (Plough, 1936; Plough and Jones, 1937, 1938). This investigation is still in progress and will be reported separately, but in connection with it specimens of all the following species of this family of ascidians were grown in the laboratory: *Perophora viridis, P. bermudensis, Ecteinascidia turbinata, E. conklini, E.* sp. nov. The fine, rapidly growing stolons of the last species appear to be a useful, if indeed not a necessary, factor in these artificial composite colonies, yet the species is undescribed. It had been collected by Professor Caswell Grave during previous seasons, and he first called it to the senior author's attention. It has been found in increasing numbers during the two succeeding seasons, and all stages in its life history have been followed. We have called it *Ecteinascidia tortugensis* after its type locality.

Accurate identification of most ascidian species often requires more than a description of the adult zooid (or blastozooid in compound forms). Some description of the larva and of stages in metamorphosis are usually needed. In compound forms a comparison between oözooid and blastozooid is desirable. These desiderata, of course, cannot be given when species are described from preserved material only, but they should be included when the study, as at the Tortugas Laboratory, is made on the living animal. Finally, the relationships of the family Perophoridae to other ascidian families have been under discussion (Van Name, 1921; Berrill, 1936) and the present species seems to throw some light on the questions at issue. Accordingly, the complete life history has been studied on living material supplemented by stained and mounted specimens. The plates and figures are entirely the work of the junior investigator (Jones). Their clarity and accuracy reduces the need of detailed description.

Acknowledgments

We wish to express our thanks to the Carnegie Institution of Washington for the rare opportunity to work at the Tortugas Laboratory, and to Professor Caswell Grave, and especially to Professor David H. Tennent, the Executive Officer, for thoughtful assistance in making our work profitable. We wish to

acknowledge also the kind assistance of Mrs. Ruth McClung Jones of Swarthmore College and Dr. Carl E. Meyer of Amherst College in sectioning and mounting preserved material to supplement our study of the living animals.

Description of Species

PEROPHORIDAE—Giard, 1872

Following Hartmeyer (1911), Van Name (1921), and Berrill (1932), we may characterize the family as follows: Colony-forming Phlebobranchia. Single animals separate, but bound to one another by creeping stolons to which they are attached. Tentacles simple. Branchial sac with inwardly projecting papillae on the transverse bars which support internal longitudinal vessels. Dorsal lamina with tongue-shaped languets, connected basally by a membrane. Stomach and intestine on left side of branchial sac, with often a globular supplementary stomach between. A digestive gland overlying the mid-intestine with a duct entering the pyloric portion. Heart on the right side of the branchial sac. Gonads in the loop of the intestine, the ovary partly encircled by a fan of testicular lobes.

Ecteinascidia—Herdman, 1880

The genus has been recently redescribed by Berrill (1932) and may be characterized as follows: Perophoridae with an elongated branchial sac bearing many (15 or more) rows of stigmata. Oviduct short and wide, discharging the eggs into the right atrial cavity, where they develop to the tadpole stage. The period of sexual maturity is followed by a period of stolon growth and budding to form colonies of blastozooids.

Ecteinascidia tortugensis species nova (plate 1)

Distinctive characters. The species is clearly an *Ecteinascidia,* as shown by the creeping stolons, the buds, and the *Ascidia*-like internal anatomy. There are 18 rows of stigmata in blastozooids. The gut is on the left side of the branchial sac and the heart on the right. It can be readily separated from the other species of *Ecteinascidia* by the widely separated dorsally placed siphons, inclined away from each other, and by the attachment of the test to rock along the ventral side. The intestine is distinctly recurved toward the stomach, and the anus is no farther anterior than the primary loop. The zooids are smaller than either *E. turbinata* or *E. conklini,* and sexual maturity is about two weeks later at the Tortugas.

Type specimen. The type colony is in the American Museum of Natural History in New York, A. M. N. H. Catalogue No. 1445. Paratypes are in the National Museum, Washington, D. C., and in the Natural History Museum at Amherst College, Amherst, Massachusetts.

Habitat

This species was first found in July 1936, attached to the under sides of slate or iron slabs on the bottom below the north coal wharf at Garden Key. Specimens have appeared in the same place in small numbers in subsequent seasons. In 1937, however, they were found in large numbers attached to the under sides of broken slabs of coral rock in the shallow channel between Bush Key and Long Key. Finally, in 1938 specimens were discovered below low-tide level on the outside of Long Key, and occasionally on the outside edge of both Bush Key and Bird Key Bank. In these situations on the southeast or open side of the reefs very few other ascidians are to be found, yet late in July 1938 this species was present in countless numbers—enough to be correctly called one of the commonest ascidians at the Tortugas. That it has not been found before in spite of frequent collecting is to be accounted for probably by its small size, its lack of strong color, and its late period of maturity. The species appears to require pure water from the open sea and prefers to live where there is constant wave action, which close attachment to the rocks makes it able to withstand.

Description of Colony and Zooids

Unlike other *Ecteinascidia* species, *E. tortugensis* has little color (pl. 1). The colonies may be spread all over the surface of an overturned rock and at first be invisible. With a lens they appear to have a slightly yellowish-green color, which may be obscured by minute fragments of limestone attached to the tests. They are usually mixed with colonies of *Perophora bermudensis,* and the colors are much the same. The individual zooids are larger than those of *Perophora,* averaging 5 to 6 mm., and they are usually more closely placed and more immovably attached. *Perophora* will move freely when the water is agitated, but *E. tortugensis* remains rigid. This character is enough to separate it from the larger orange-colored *E. turbinata* and from the bright green *E. conklini.* Outline drawings of the various species are shown in text figure 1.

The mode of attachment of *E. tortugensis* is peculiar, resembling some of the larger solitary species of *Ascidia.* It has no stalk, though stolons grow off from the posterior ventral border. The test, however, adheres to the adjacent rock by at least half its length along the ventral side. This makes it extremely difficult to remove from the rock uninjured. We have found that the only practicable method of securing specimens for study or growth in the laboratory is carefully to insert a scalpel under the test, and, working under a lens, slowly to advance it against the rock. The test is very thin along the area of attachment and is easily broken. Young budding colonies have the zooids scattered at intervals of 5 to 10 mm., connected by the thin branching stolons (text fig. 2). Older colonies may have the zooids so crowded as to form a mat with their tests adherent to each other as well as to the rock.

Another character which distinguishes this species from other *Ecteinascidia* is the position of the siphons. These are placed on the dorsal side above the dorsal lamina, and opposite the area of attachment. The oral siphon is anterior but ordinarily opens upward, while the atrial siphon is placed dorsally about two-thirds of the way toward the posterior end of the body and is ordinarily

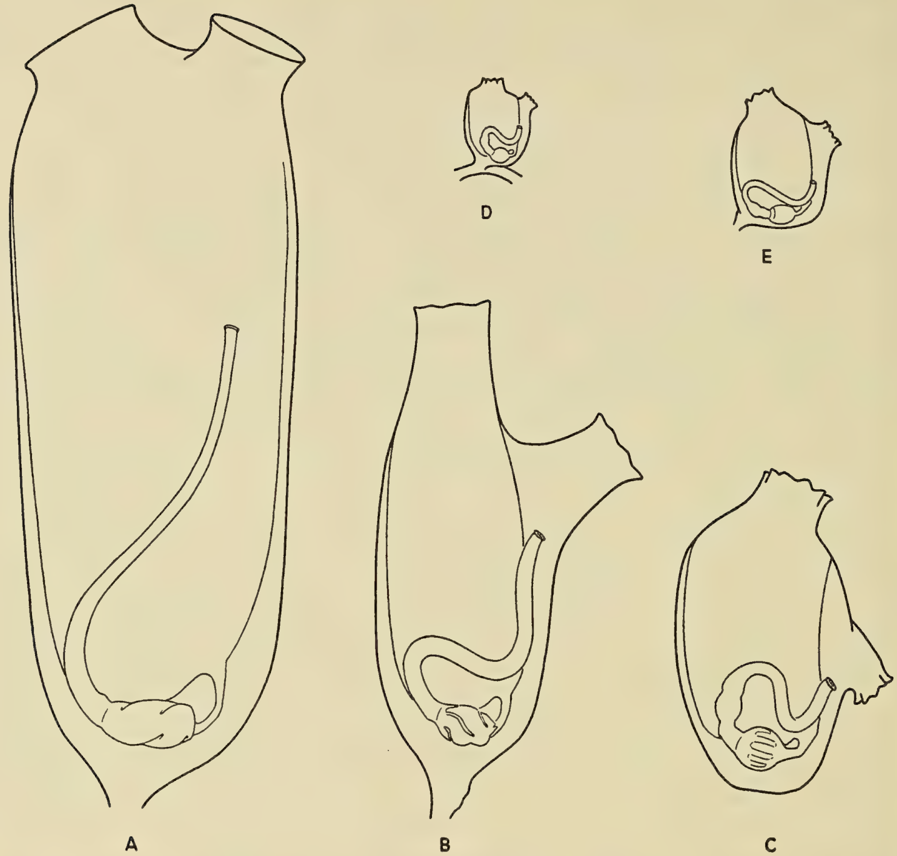

Fig. 1. Comparison of Perophoridae found at Tortugas, drawn to same scale. A, *Ecteinascidia turbinata;* B, *E. conklini;* C, *E. tortugensis;* D, *Perophora viridis;* E, *P. bermudensis.*

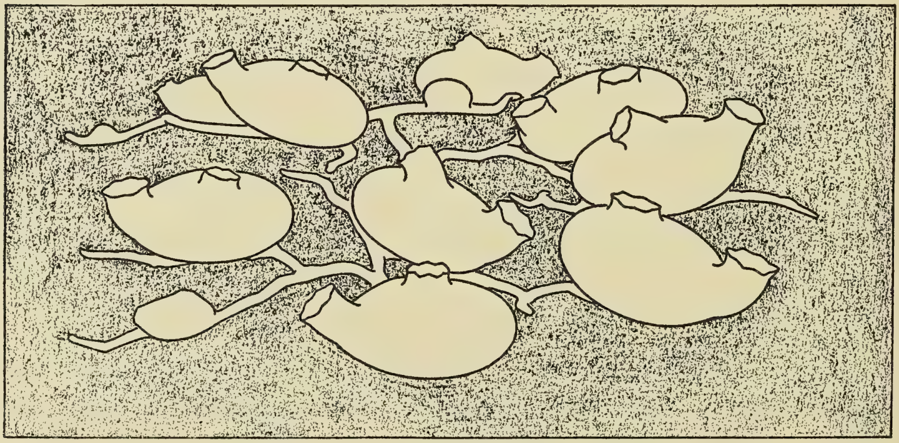

Fig. 2. Colony of *Ecteinascidia tortugensis* on slab of coral rock

directed away from the oral one. The outlines in text figure 3 show several common arrangements. Such wide separation of the siphons is never found in *E. turbinata,* though an approach to it may occasionally occur in *E. conklini.* It is a common arrangement in solitary forms of Ascidiidae, as *Ascidia sydniensis.*

Internal Anatomy

When a zooid is removed from its test its characters as a typical *Ecteinascidia* appear. There are only slight differences from *E. conklini.* External views are shown in plate 1, and the internal anatomy, somewhat schematized, is shown in plate 2, figures 1, 2, 3. The tentacles are simple and filiform, about 30 in number. In mature blastozooids there are 18 rows of stigmata in the branchial sac. The dorsal tubercle is V-shaped and the dorsal lamina is a continuous membrane with V-shaped languets at each transverse bar (pl. 2, figs. 4, 5).

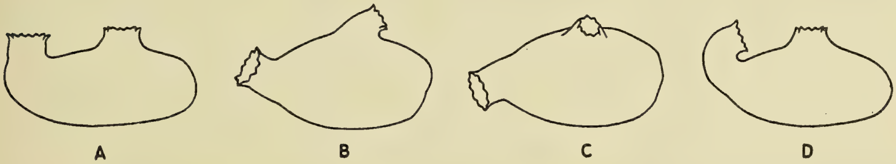

FIG. 3. Various forms of zooids of *Ecteinascidia tortugensis.* The two at the left are the most common.

Stained and cleared specimens show a characteristic duct leading from the dorsal tubercle to the subneural gland and ganglion (pl. 2, fig. 4). The length of the latter is a little less than that of 2 rows of stigmata. Characteristic internally projecting papillae arise from the inner sides of the transverse bars between every second stigma and the next stigma in each row, and these join to form typical longitudinal vessels (pl. 2, fig. 6). In young zooids only are the longitudinal vessels incomplete.

The method of food collection in the branchial sac can be demonstrated easily by adding powdered carmine to the water. A mucus column stained red with entangled particles lies in the dorsal third of the branchial sac and passes from the region of the oral siphon directly into the esophagus (pl. 3, fig. 1). This continuous stream of particles is the food supply. The mucus appears to be secreted in the endostyle and carried dorsally by ciliary action on the transverse bars, where the particles sifted out by the stigmata become entangled in it. In the region of the dorsal lamina the mucus tends to drop off the transverse bars all along the branchial sac. Thus the column is formed which is drawn continuously into the esophagus. The water current, of course, passes through the ciliated stigmata and out the atrial siphon. The course of the carmine particles can be followed from the esophagus, through the stomach, out the pyloric valve into the globular enlargements of the intestine. There they are formed into pellets which are finally expelled from the anus.

The digestive tract lies entirely on the left side of the branchial sac. The funnel-shaped esophagus opens out of the latter just below the dorsal lamina and slightly to the left. It passes into the globular stomach, and the latter opens by a pyloric valve into the intestine. At about the same point the duct of the pyloric or digestive gland enters. The separate lobes of the gland overlie the middle portion of the rectum, and branches from each join to form the duct which crosses the loop under the sperm duct (pl. 3, fig. 2). The mid-intestine is usually constricted to form two globate portions before passing into the rectum. The latter is sharply recurved close to the stomach, forming a secondary loop. It thus bends into the atrial cavity above the dorsal lamina, where the anus opens about as far anterior as the primary loop of the rectum. This constitutes an easy distinguishing feature from *E. turbinata* and *E. conklini,* in each of which the anus is much farther anterior, and the secondary loop is not so marked (see text fig. 1).

The heart lies, as in other Perophoridae, on the right side at the posterior end of the branchial sac. It is continuous anteriorly with the subendostylar vessel. This is connected with the vessels in the transverse bars and these in turn with the inner longitudinal vessels. The blood finally enters the vessel above the dorsal lamina, which runs to the gut and from there back to the posterior end of the heart. At this point the large ventral stolonic vessel also joins the heart (pl. 2, figs. 1, 2, 3). The characteristic reversal of the heart beat occurs about every 2 minutes at 28° C.

Finally, the gonads lie within the loop of the intestine as in the other Perophoridae. The testicular lobes are larger and less numerous than in *E. turbinata.* They lie in a crescent close to the intestine and from them vasa efferentia converge toward the center to form a large sperm duct (pl. 1; pl. 3, fig. 2). The latter bends dorsally with the rectum and opens in the atrial cavity just posterior to the anus. The more compact ovary is separate, but partly encircled by the testis. The short, wide oviduct is difficult to see in the live animal unless an egg is passing through it. Sections show that it is similar to the oviduct of *E. turbinata* as described by Berrill (1932). It passes close to the sperm duct dorsally but fails to follow its anterior curve, and just beyond the dorsal lamina it opens to drop the mature eggs into the posterior right atrial cavity (see pl. 1; pl. 3, fig. 3).

Development

The development of the egg of *Ecteinascidia tortugensis* has been studied in some detail both alive and in sections. A brief review of the process will be given here, and reference should be made to plate 4. The eggs are fertilized in the atrial cavity and development proceeds to the tadpole stage within the chorionic membrane. By this time the embryo has been pushed anteriorly close to the region of the atrial siphon by the pressure of more eggs released from the oviduct (see pl. 3, fig. 3). As noted by Berrill (1935*a*) for *E. turbinata* and *E. conklini,* the tadpole is released by rupture of the membrane and eventually expelled into the sea water by contraction of the atrial siphon. By opening the atrial cavity, eggs and embryos in all stages can be secured for study. If these are placed in stender dishes in which the water is changed several times a day, development proceeds normally. The precautions used by Berrill (1935*a*) for keeping a continuous stream of water flowing over the eggs are at Tortugas unnecessary.

The undivided egg of *E. tortugensis* measures about 0.5 mm. in diameter. It is nearly as large as that of *E. conklini* and its development is similar. (See also Simkins, 1925, for *E. turbinata.*) The sea water at Tortugas during late July and August averages 28° C., and naturally the development proceeds much more rapidly than that recorded by Berrill for the other species at 16° C. The first cleavage occurs about 4 hours after the egg leaves the oviduct, and succeeding cleavages at intervals of slightly less than 2 hours. Gastrulation is embolic, as noted by Berrill, and begins at about 24 hours (pl. 4, figs. 1, 2, 3, 4). The tadpole is fully formed within the membrane at 48 hours, and it ordinarily hatches on the third day. It does not swim immediately, but remains quiescent with an occasional twitch of the tail until the fourth day, during which time it normally remains within the atrial cavity. The heart begins to pulsate during this period (pl. 4, figs. 5, 6, 7, 8).

When freed, the tadpole swims actively for 2 to 4 hours and sometimes much longer. The structure of the larva of *Perophora* has been described in detail by Grave and McCosh (1923), and certain features of that of *Ecteinascidia* by Berrill (1932, 1935*a*). Since no important differences were noted between the larvae of *E. conklini* and *E. tortugensis,* no description will be given and reference may be made to plate 4, figure 9 for details. One correction in Berrill's account is worthy of note. In the swimming larva of *E. tortugensis* the branchial sac is fully formed and contractile though the siphons are not open. At first the stigmata are not visible in living specimens, but somewhat later in transparent larvae it is plain that 4 rows of definitive stigmata are formed, and in cleared and stained specimens this observation was confirmed (pl. 4, figs. 10, 11). Since Berrill gives 6 rows as the number in *E. conklini,* larvae of the latter species were examined at the same stage and here again it was demonstrated with certainty that 4 rows are present. This observation actually adds support to Berrill's thesis (Berrill, 1935*a,* p. 317) that development of the stigmata in the Perophoridae parallels that in the Styelidae in the relation between yolk volume and differentiation. In *E. tortugensis* and *E. conklini* a fifth and then a sixth row of stigmata are added by divisions of the anterior rows, making 6 in all (see pl. 4, fig. 11). In *E. turbinata* 12 rows are present in the swimming larva, but the condition in younger tadpoles has not been carefully studied. The observation on *E. tortugensis* and *E. conklini* establishes more firmly the relationship between *Ecteinascidia* and *Perophora,* since the latter also possesses 4 rows of definitive stigmata in the oözooid from the beginning.

The later history of the larva of *E. tortugensis* resembles closely that of *E. conklini.* It attaches at the region of the adhesive papillae, and within a few hours the notochord, nerve cord, and other tissues of the tail begin to be resorbed. This appears to be a variable process, since for 24 hours after attachment the tails of some larvae have been found capable of independent movement. Eventually, however, only the clear test covering of the tail remains (pl. 4, figs. 11, 12). During the first day after attachment also the siphons break through and the gut becomes functional, while the extensive larval brain vesicle becomes relatively reduced to the ganglion and subneural gland between the siphons.

From 1 to 2 days after attachment, three or four ampullae grow out on either side of the adhesive papillae. These soon show a median longitudinal septum and become vascularized. These ampullae are holdfasts for the young oözooid. They may eventually grow out as stolons, but such stolon growth has not occurred in our specimens kept under observation for as long as two weeks after attachment of the larva. Oözooids of *E. turbinata* and *E. conklini* have been observed for as long as three weeks without stolon growth. Berrill (1935*b*, p. 358) states for the Perophoridae, ". . . the young oözooids born during the summer bud rapidly until the following spring, when the process dwindles, and the zooids attain sexual maturity." We have not been able to confirm this for *Ecteinascidia,* and it may be that oözooids grow slowly without budding until the following spring, when sexual maturity is attained.

The formation of additional rows of stigmata apparently occurs by division of the 6 rows found in the metamorphosed oözooid. Stages of 8 and of 12 rows were seen in larvae of *E. conklini* which had been observed for 10 and 14 days after metamorphosis, but these stages have not been continuously followed in *E. tortugensis.* Soon after the growing out of the ampullae, about three days after attachment, young oözooids of *E. tortugensis* can be distinguished from similar stages of *E. conklini.* The former become more and more attached along the ventral side of the test, with the atrial siphon pointing upward and away from the oral. Young oözooids of *E. conklini,* however, continue to grow in the upright position with both siphons at the oral (anterior) end and pointing in the same direction (see text fig. 1).

Budding and Colony Formation

Stolon growth and bud formation in the Perophoridae have been studied by many investigators over a long period (see Lefevre, 1898; Huxley, 1921; Beers, 1923; Brien, 1927; Deviney, 1934; Berrill, 1935*b;* Plough, 1936; Plough and Jones, 1937, 1938). The interest in the process lies in the fact that the growing stolons contain mesenchyme and blood cells only, with a single layer of covering ectoderm. At intervals an enlargement appears (a bud) consisting of a thickened area of ectoderm and an inner vesicle derived entirely from the mesenchyme of the stolonic septum, and from this a new zooid (blastozooid) is differentiated. The ectoderm forms only the external layer and mesenchyme all the other organs. Thus in asexual development a single undifferentiated embryonic tissue is able to form all adult organs, even though in the development of the egg, experiments on other ascidians have demonstrated that the mesenchyme has no such potency. In view of this interesting situation, the development of the blastozooid of *Ecteinascidia tortugensis* has received special study (see pl. 5).

Sexually mature zooids (blastozooids) of *E. tortugensis* have been removed from slabs of rock collected in early July on Long Key. Kept in small stender dishes on the laboratory water stand, such zooids shed tadpoles for 3 or 4 days, and then the gonads appear to recede, finally ceasing to produce eggs. With the cessation of sexual activity, stolons begin to grow and to branch along the bottom of the dish (see pl. 1). Eventually one or more enlargements (buds) appear along the stolon—never at the tip—and after about 6 days these are differentiated into functional blastozooids. The process is illustrated in plate 5. The heart is differentiated precociously and begins to beat on the third day. The stigmata are not visible from the outside until after the siphons have broken through the test wall, but as the zooid becomes larger and more transparent they appear. Cleared and stained specimens show that 10 to 12 rows of stigmata break through simultaneously in the buds, and sections confirm these counts. As the buds elongate, the more anterior stigmata also elongate, and eventually the latter divide to form 2 rows. The adult number of 18 rows is finally attained in this way.

Comparisons with buds of about the same stage in *E. conklini* and *E. turbinata* have been made. In each of these also the initial number of rows of stigmata is less than the number in the adult. When the stigmata first break through there are 12 rows in *E. conklini* and 15 in *E. turbinata* (see also Beers, 1923). The final numbers of 20 in *E. conklini* and 30 in *E. turbinata* are formed in the way described above. Finally, buds of *Perophora bermudensis* (blastozooid number 5) were studied. Here 4 rows break through at first and later the most anterior row divides to make 5. It is thus a general character of the Perophoridae that the number of rows of stigmata which first appear in the developing blastozooid approaches, but does not equal, the number in the adult (except in *Perophora viridis,* where the adult number is 4). This is in marked contrast to the condition in the developing oözooid, as noted in the preceding section. (See table 1.)

TABLE 1. *Number of rows of stigmata in various stages of Tortugas Perophoridae*

Species	Tadpole larva	Oözooid	Early blastozooid	Adult blastozooid
Ecteinascidia turbinata	12	24–28	15	28–30
Ecteinascidia conklini	4	18–20	12	20
Ecteinascidia conklini var. minuta	?	?	10	15
Ecteinascidia tortugensis	4	15–18	10	18
Perophora bermudensis	4	4	4	5
Perophora viridis	4	4	4	4

Regression and Resorption of Blastozooids

In colonies of *Ecteinascidia tortugensis* growing in dishes on laboratory tables or in the tanks, especially when the food supply is limited, it has been observed frequently that one of the larger zooids will grow smaller day after day. Closer examination always discloses that at the same time its stolon system is growing and usually one or more buds are increasing in size at the normal rate. This is exactly the same process as that described by Huxley (1921) for *Perophora.* The stolons and buds grow at the expense of the large zooid, which becomes progressively dedifferentiated. The anterior rows of stigmata are resorbed first, so that a stage with a reduced number of stigmata may be seen, but once started the zooid progressively diminishes until only a small knob of undifferentiated tissue remains. The histology of this and other processes, such as bud formation, in *E. tortugensis* has received some study. In general the results are in accord with those reported by Deviney (1934) and Berrill (1935*b*).

Relationships of Ecteinascidia tortugensis and of the Perophoridae

Hartmeyer (1911) lists four genera making up the family Perophoridae: *Perophora, Ecteinascidia, Perophoropsis,* and *Sluiteria.* Of these only the first two are known on the coast of North America.

Perophoropsis was described from Banyuls in the Mediterranean by Lahille (1890), and apparently has not been seen since. According to the description and figure the only characters distinguishing it from *Ecteinascidia* are: 15 to 16 rows of stigmata without vascular papillae and "a tendency of the stolons to atrophy so that the zooids appear isolated." Comparison with our table 1 shows that the first character is a transitional stage in the oözooid or the blastozooid of either *E. conklini* or *E. tortugensis.* In a group of oözooids of either species no stolons would appear. Berrill (1932, fig. 3) figured a young oözooid of *E. conklini* with 10 rows of stigmata which he believed resembled *Perophoropsis.* These facts, together with our figures, make it certain that *Perophoropsis* Lahille (1890) was in reality a larval stage (oözooid) of *Ecteinascidia* Herdman (1880). The genus *Perophoropsis* should accordingly be dropped from the literature of the family.

Sluiteria, on the other hand, has a cellulose tunic cut by folds, which end in conical papillae, and is thus widely different from the other genera.

In the adult anatomy, especially in the secondary loop of the intestine, in the number of rows of stigmata, and in the development of larva and bud, *E. tortugensis* takes an intermediate position in the family. *E. turbinata* shows greater extension of the branchial sac, while at the other extreme *Perophora* is abbreviated (like the *E. tortugensis* larva). The fact that other species (as *E. conklini*) show this same intermediate condition may suggest that such species exhibit the more primitive condition from which the more extended and the more abbreviated types have been independently derived.

In general, investigators of the structure and taxonomy of the Ascidiacea have placed the Perophoridae in the order Phlebobranchia close to the Ascidiidae, and Van Name (1930) includes them within this family. As a result of a series of careful and detailed studies ranging over the whole group, Berrill (1935*a*, 1935*b*, 1936) concludes that they should be set apart by themselves as a separate branch derived independently from the primitive Cionidae. With much of Berrill's argument we are in agreement, and especially with his suggestion of the need of a reclassification of the whole group based on recent studies. Two facts in our study of *E. tortugensis,* however, cause us to prefer the earlier interpretation of the relationships of the Perophoridae. First, the number of rows of stigmata in the larva is 4 increasing to 6 as in the Ascidiidae and Botryllidae. Second, the growth habit of *E. tortugensis,* attached along the ventral side of the tunic, is exactly similar to that of many species of *Ascidia* and the Styelidae. The stolons grow off all along the attached tunic instead of from a stalk at the aboral end. These two points of resemblance in a primitive member of the Perophoridae in our opinion favor one of Berrill's alternative suggestions, namely (1936, p. 60) that they represent "an early step in the change from the cionid to the ascidiid type."

SUMMARY

A new member of the family Perophoridae (Ascidiacea Phlebobranchia) is described and named *Ecteinascidia tortugensis* from its type locality, the Dry Tortugas Keys, Florida. It is shorter than other *Ecteinascidia,* lies on the ventral side attached along the test, has the siphons on the dorsal side widely separated and opening in opposite directions, and possesses a marked secondary loop in the intestine.

This species reaches sexual maturity early in July at the Tortugas, about two weeks later than *E. conklini.* A brief account of the development is given. The tadpole larva contains 4 rows of stigmata at first, with 2 additional rows added by the time of metamorphosis.

Stolon growth and budding begin after the period of sexual maturity, and colonies form a mat over the under sides of coral rock. Some account of the formation of the blastozooid is given.

The structure and growth habits of *E. tortugensis* indicate that it is intermediate between *E. turbinata* and *Perophora.* They suggest a relationship of the Perophoridae with the Ascidiidae.

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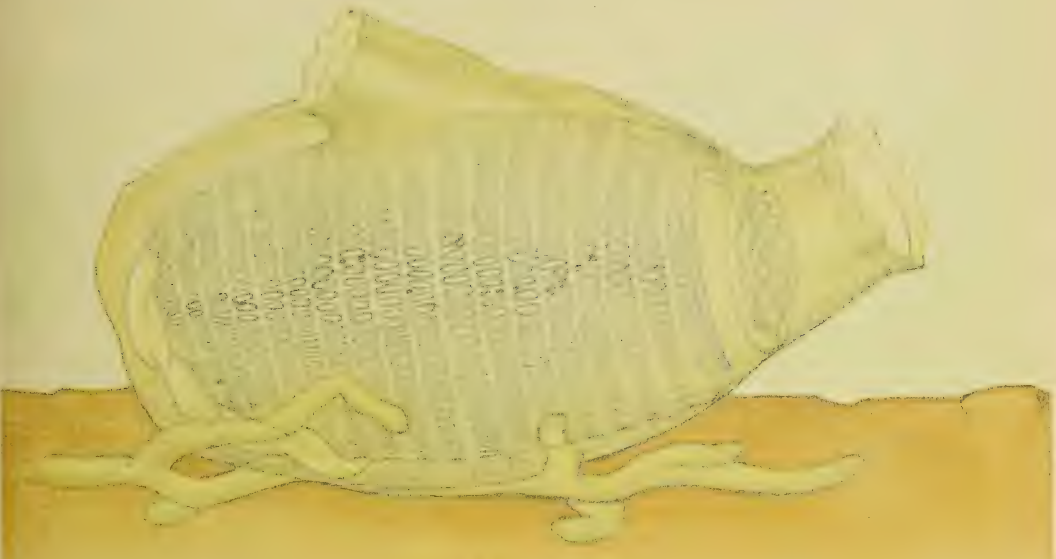

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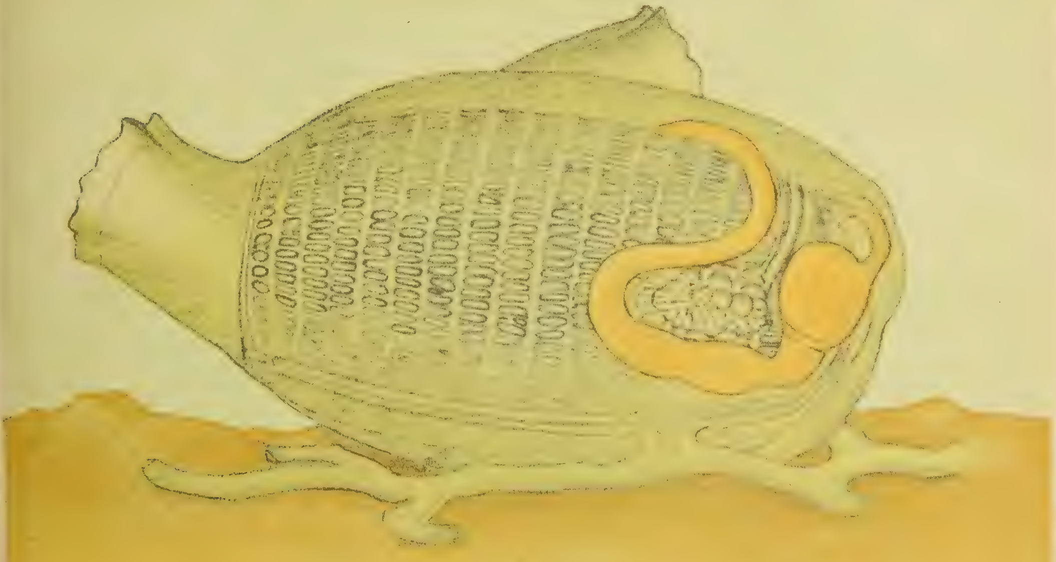

Left side

ECTEINASCIDIA TORTUGENSIS.

External views of *Ecteinascidia tortugensis* from right and left side. Figures are composites showing mature gonads, with sperm and oviduct within the loop of the intestine (in lower drawing), and also growing stolons. These are not ordinarily found in one individual at the same time. × 30.

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PLATE 2

FIG. 1. Optical section of an immature zooid of *Ecteinascidia tortugensis* from the right side, somewhat simplified and schematized. Oral siphon above and atrial siphon at the left. The blood is green, and the endostyle yellow. Heart is dotted. × 10.

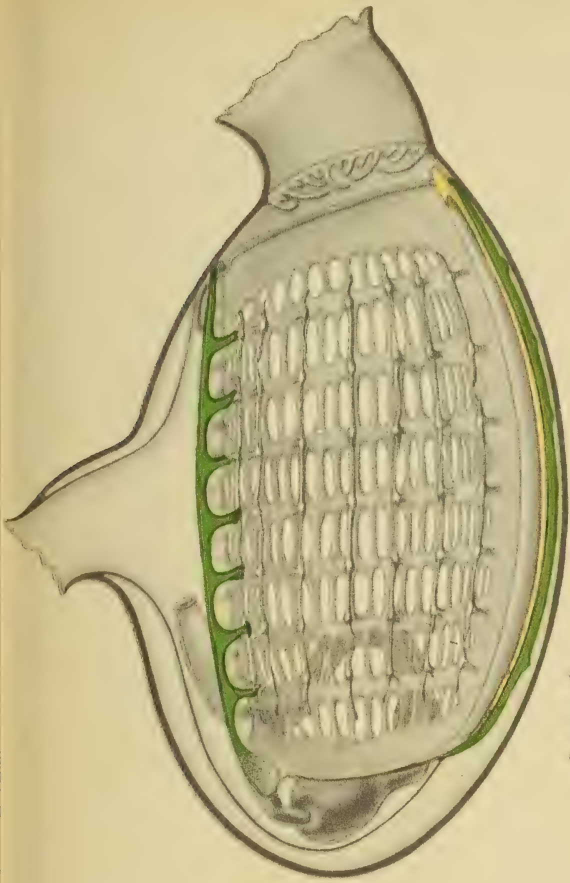
FIG. 2. Schematized section of the upper (oral or anterior) half of the same zooid looking from the mid region toward the oral siphon. × 10.

FIG. 3. Schematized section of the lower (aboral or posterior) half of the same zooid looking from the mid region toward the stomach. The opening of the esophagus is the dark circle at the left center. × 10.

FIG. 4. Enlarged detail of the ganglion, subneural gland, and duct to the dorsal tubercle as seen from above in stained specimens. × 20.

FIG. 5. Enlarged detail of the anterior part of the dorsal lamina, showing languets and dorsal tubercle as seen from below. × 20.

FIG. 6. Detail of a small part of the branchial sac seen from within, showing the internal papillae fusing to form the inner longitudinal vessels. × 20.



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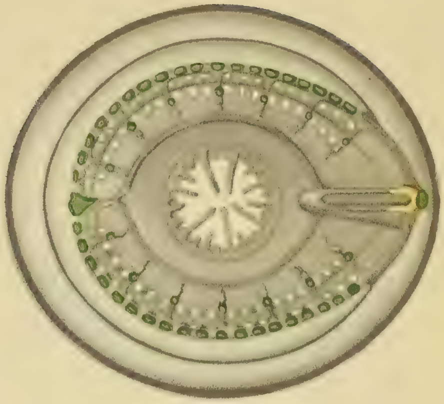

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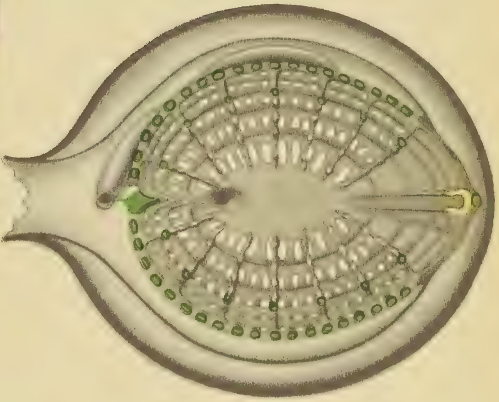

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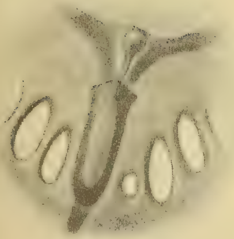

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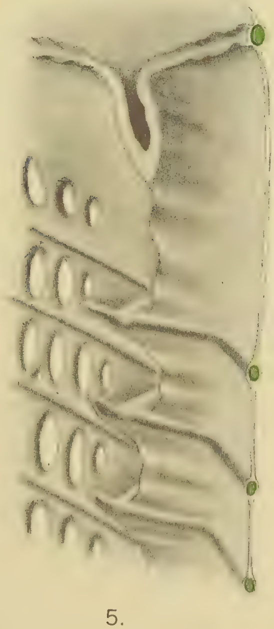

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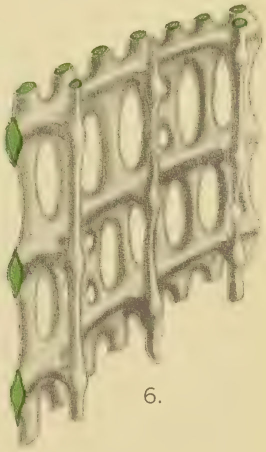

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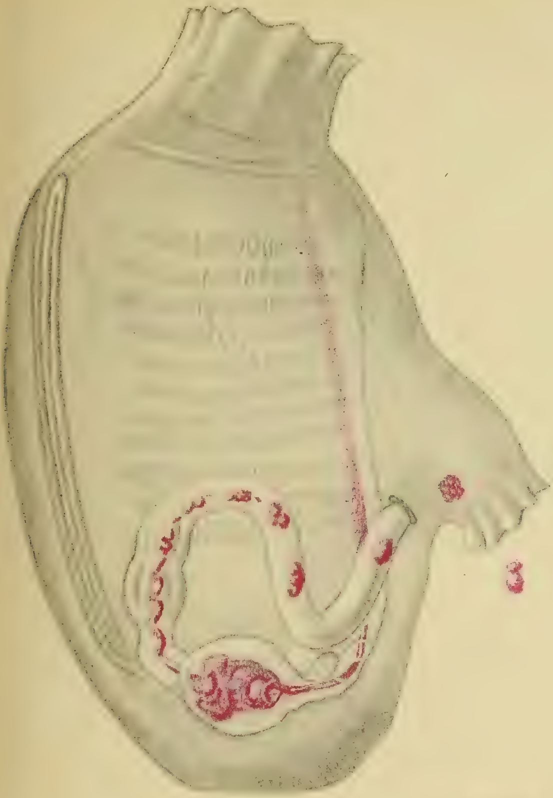
PLATE 3

FIG. 1. Living zooid of *Ecteinascidia tortugensis* which has been fed a suspension of carmine particles, showing the mucus column being drawn into the esophagus, and the course of the carmine through the digestive tract. × 9.

FIG. 2. Details showing the intestinal loop enclosing mature gonads (testis outside, ovary at the center, of the crescent), and the sperm duct. The oviduct is not visible. In addition note the duct from the digestive gland crossing under the sperm duct and entering the pyloric region of the intestine, just beyond the stomach. Both tunic and mantle have been removed. × 9.

FIG. 3. Sexually mature zooid from above and to the right, showing developing eggs and larvae within the right atrial cavity. The end of the oviduct is shown at the lower left. × 9.



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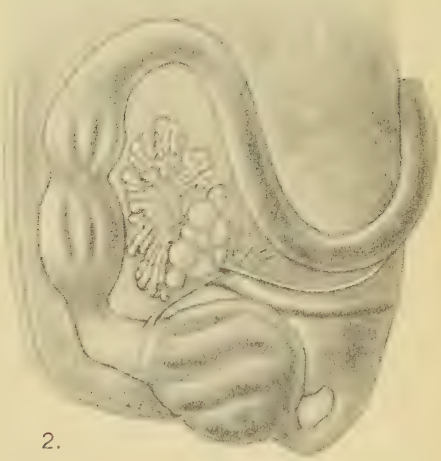

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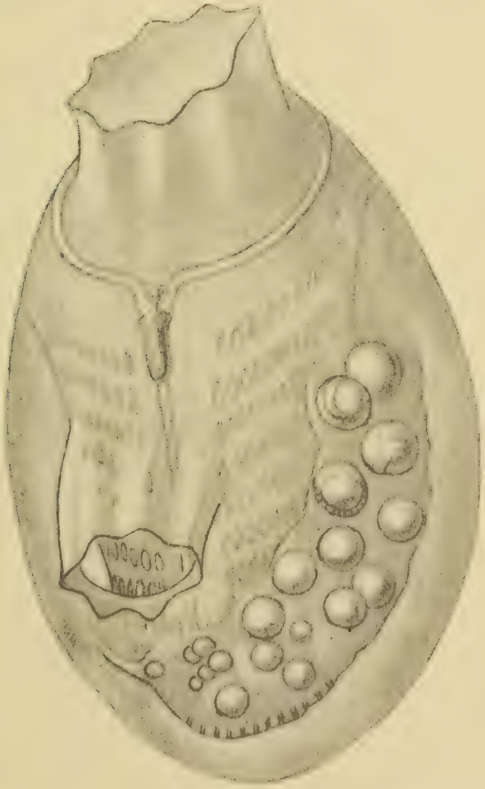

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PLATE 4

Development of the egg and metamorphosis of the tadpole in *Ecteinascidia tortugensis*. All figures × 60. The time intervals are indicated in the text.

FIG. 1. Undivided egg within the chorion.

FIG. 2. Four-cell stage from animal pole.

FIG. 3. Sixteen-cell stage from vegetative pole.

FIG. 4. Gastrula.

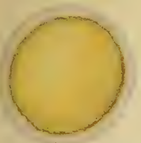
FIGS. 5, 6, 7. Successive stages in formation of tadpole within the chorion.

FIG. 8. Tadpole larva at the time of hatching. Note the notochord, brain vesicle, and suckers, or adhesive papillae.

FIG. 9. Swimming tadpole larva. Note siphons at the left (not yet functional) with brain vesicle between. Above are the suckers with ampullae growing out on either side. The heart is already visible and beating.

FIG. 10. Larva shortly after attachment. Metamorphosis has begun: notochord and tail musculature has broken down and is being resorbed. The siphons have enlarged and are about to break through. The four rows of stigmata begin to be visible through the wall.

FIGS. 11, 12. Successive stages in the completely metamorphosed oözoite. Tail tissue is resorbed completely leaving only the test. The stigmata increase to six rows. The ampullae grow out as incipient stolons which do not increase beyond this point until after a long interval of growth of the zooid.



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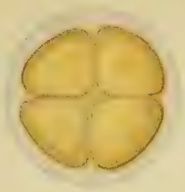

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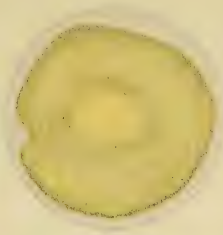

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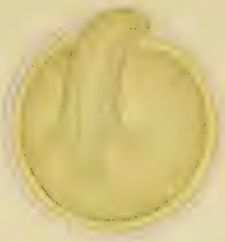

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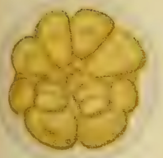

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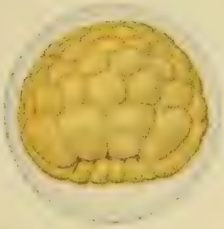

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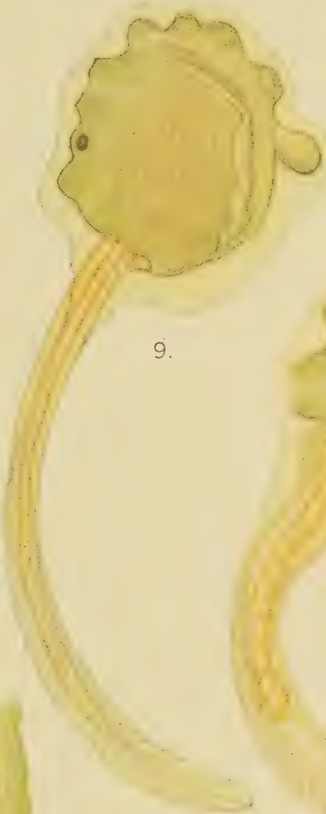

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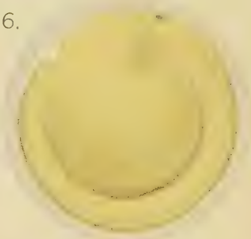

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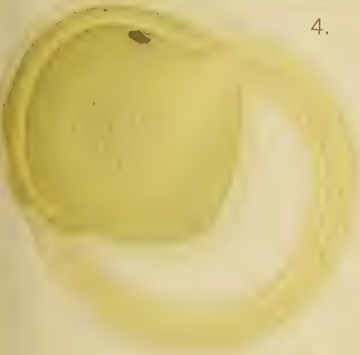

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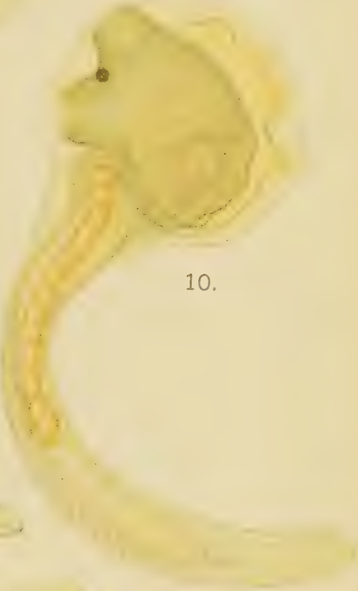

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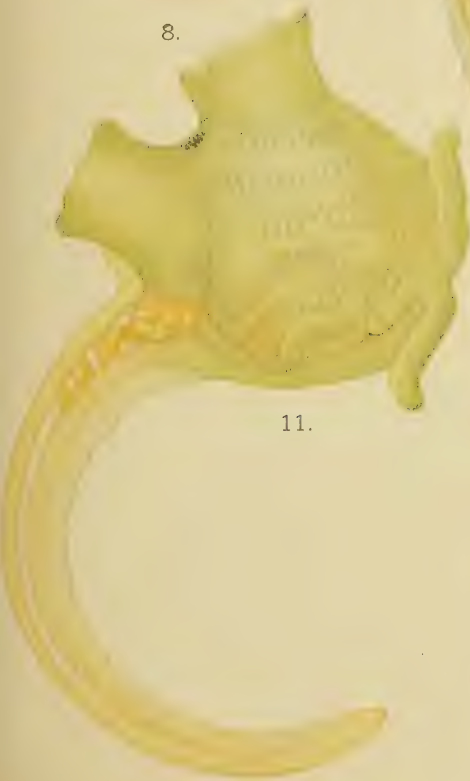

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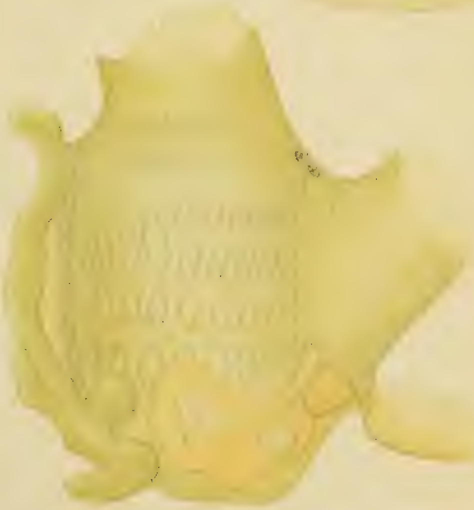

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PLATE 5

Asexual reproduction or budding in living *Ecteinascidia tortugensis.* All figures × 60.

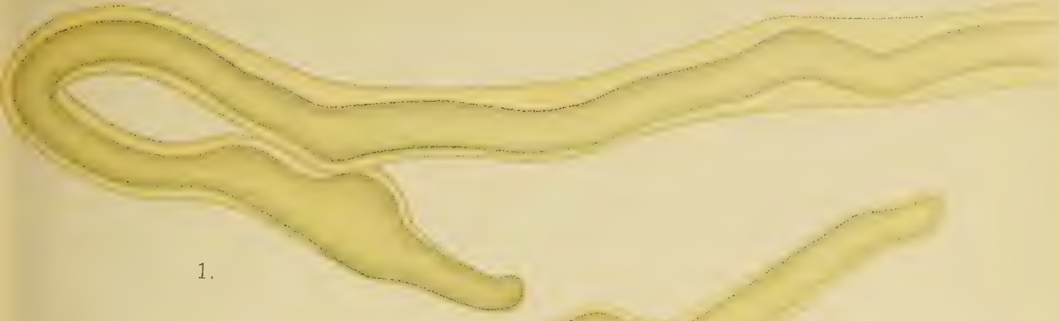
FIG. 1. Elongated stolon grown out from a zooid which has passed sexual maturity. A beginning bud appears just short of the growing point.

FIGS. 2, 3. The bud increases in size and is gradually separated from the stolon (2 to 3 days old).

FIG. 4. The bud is completely differentiated, the heart is beating, but the siphons have not yet broken through (4 days old).

FIG. 5. The bud is now a functional blastozoite. Stained specimens show about ten rows of stigmata at this stage (5 to 6 days old). Some of the stolon has been resorbed.

FIG. 6. A blastozoite 6 to 8 days old. Thirteen rows of stigmata are visible. Structure as in the adult.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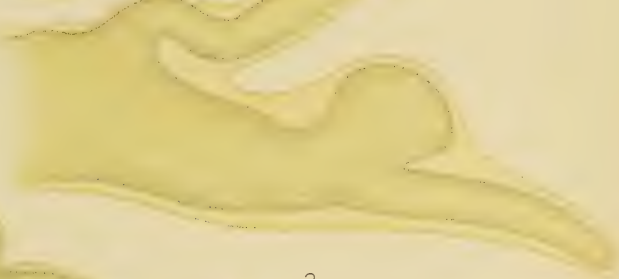
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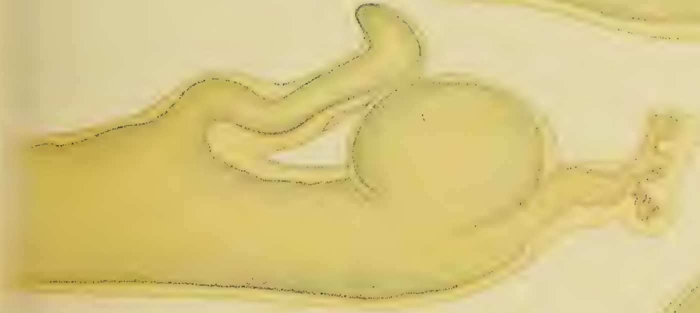
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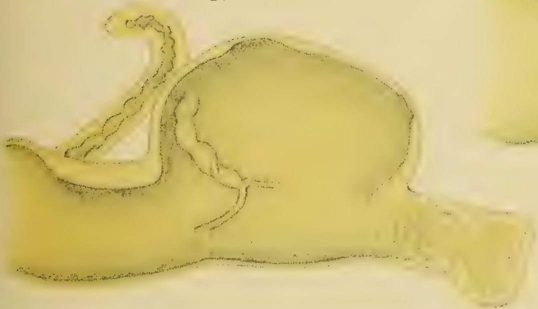
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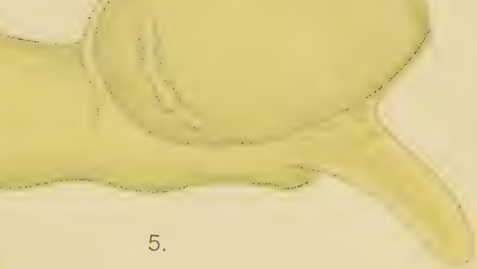
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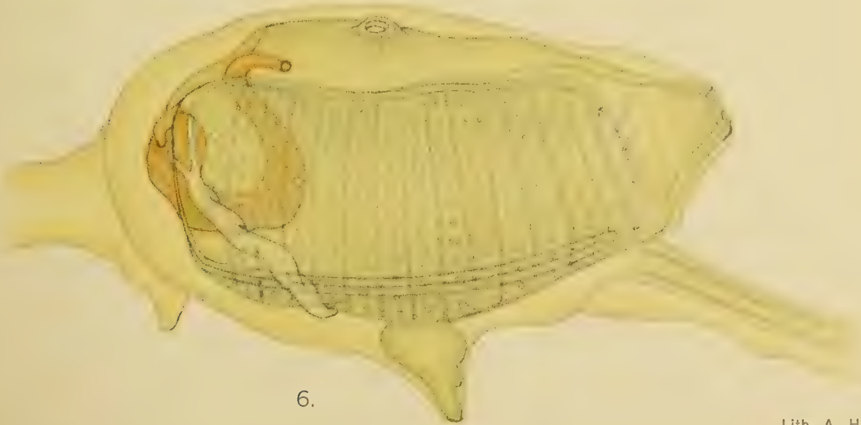
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PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

III

Symmetry in Normally Asymmetrical Crustacea

By Hugh H. Darby

Department of Biological Chemistry, College of Physicians and Surgeons,
Columbia University

With one plate

[ISSUED October 31, 1939]

Symmetry in Normally Asymmetrical Crustacea

In 1934, the writer reported the experimental production of symmetrical specimens of *Crangon armillatus* (Milne-Edwards) from naturally occurring asymmetrical forms. In the years following, in the course of investigations on the mechanism of chela growth in these and similar crustacea, careful watch has been kept for naturally occurring symmetrical forms for comparison with the experimentally produced animals. On June 10, 1937, while making a routine collection on Long Key flats, Tortugas Islands, Florida, the writer found such an animal—the only one among several thousands collected for experimentation. (Plate 1.)

Coutière (1899) in his work on the Alpheidae figured an "abnormal" symmetrical *Crangon* with a pair of large claws (snap claws) but offered no explanation for its occurrence. Calman (1906) published photographs of a similar case of symmetry in *Homarus gammarus,* which supported the earlier statement of Herrick (1895) that occasionally such animals were to be found in *Homarus americanus.* The writer found similar conditions in the fiddler crab, *Uca pugilator,* and reported them with photographs in 1935.

There is now a good deal of information bearing on the mechanism of chela production which helps to explain the occurrence of these unusual animals. First it must be recognized that the development of the highly modified ("large") claw is a secondary sexual character. This is especially clear in the fiddler crab, where, normally, only the male has a large claw. In *Crangon armillatus,* both sexes have one claw modified, but the degree of modification is noticeably greater in males than in females. In *C. heterochelis* males, both claws are modified, one much more than the other; whereas in females only one is modified, and to a smaller extent (Wilson, 1903; Dawes, 1934). Inasmuch as these animals exhibit a greater capacity for claw modification in males than in females, it is significant that both Calman's lobster and the writer's recently discovered *Crangon* were of male sex.

The original method of producing two large claws experimentally (Darby, 1934, 1935*b*) involved the removal of both claws with a definite interval of time between them. As these animals are continually fighting in their natural habitat, and are frequently encountered with a regenerating claw, it may be assumed that the loss of a claw is a perfectly natural phenomenon. However, the loss of both claws with a definite time interval between the two is highly improbable in the natural state.

In 1935, while investigating the effect of temperature on regeneration, the writer obtained increased production of animals with two large claws by subjecting them to temperatures of 35° C. It is of interest, therefore, that this specimen was found on an unusually shallow mud flat where the tidal pools frequently reached 40° C.

There is still another circumstance under which a pair of large claws may occur. Herrick stated that there is a hereditary tendency within a brood to have the modified claw on a particular side. This is hard to understand, as Herrick himself found equal numbers of right- and left-handed adult *Crangon*. The latter has also been the experience of Przibram, Wilson, Zeleny, Brues, Dawes, and the writer. Out of many hundreds of animals kept under close observation for long periods, only one, in the writer's experience, showed any preferential tendency. This *Crangon* had its right snap claw removed experimentally, and at molting reversed sides in the usual way, showing now a left snap claw and a right pinch claw. But on the second molt, the animal showed two snap or large claws. No other animal had done this, therefore a close watch was kept on it. At the third molt the left snap claw was imperfectly developed, while the right snap claw was as good as the snap claw originally removed from that side. On the fourth molt it had reverted completely to a right-handed form with a pinch claw on the left. This animal had a distinct constitutional tendency to one side, as some species of hermit crabs have.

As no information was given by Coutière or Calman regarding the environmental conditions under which their unusual specimens lived, there is no possibility of accounting for their occurrence. For the case reported here, high temperature seems to be the most probable explanation.

The animal went through one molt in the laboratory before being killed. The claws resembled those produced experimentally in all particulars. The specimen is now in the collection of the Smithsonian Institution.

Summary

A symmetrical specimen of *Crangon armillatus* was found in nature with two snap claws. These claws differed in no way from snap claws produced experimentally and reported previously.

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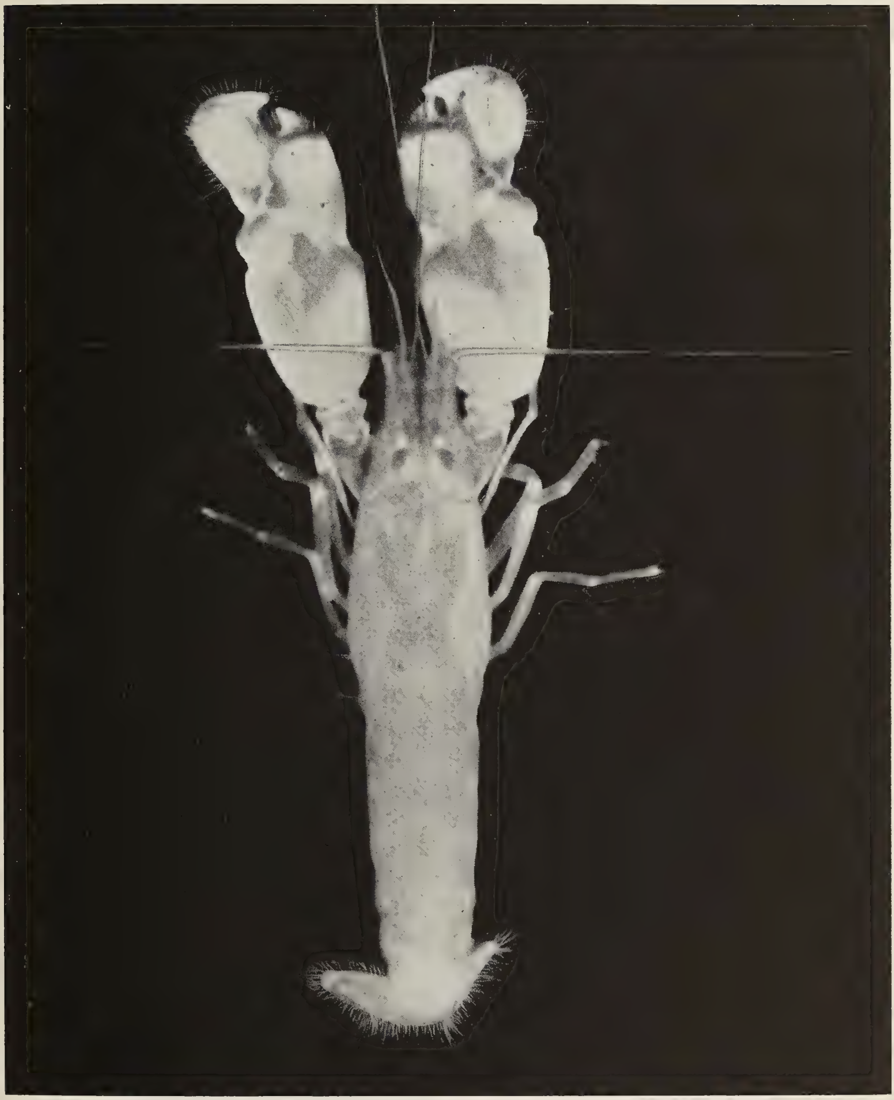

Symmetrical male *Crangon armillatus,* collected June 10, 1937, Long Key flats, Tortugas Islands

PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

IV

The Effect of Light on the Inherent E.M.F. of Valonia ventricosa

I. Intensity and Time Relations

By Gordon Marsh

Zoological Laboratories, State University of Iowa

With five text figures

[ISSUED October 31, 1939]

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The Effect of Light on the Inherent E.M.F. of Valonia ventricosa

I. Intensity and Time Relations[1]

INTRODUCTION

The interpretation of the electrical changes produced in green plants by light has been retarded by the confused nature of the published results. The favored technique of the earlier workers (Bose, 1907, 1921, 1924; Haake, 1892; Klein, 1898; A. D. Waller, 1900*a,* 1900*b;* J. C. Waller, 1925, 1929) was the measurement of the potential difference between illuminated and unilluminated portions of a leaf, usually without regard to the orientation of existing potentials or their distribution within the tissue. The illuminated portion was sometimes positive, sometimes negative to the unilluminated region, although the orientation was consistent for a given material. Sheard (1929, 1930) irradiated entire leaves of sunflower and poinsettia and found the original negativity of the apex to the stem to be decreased or reversed.

In order to interpret electrical changes in a tissue in terms of some underlying process it is essential to obtain precise information concerning the distribution of E.M.F. within the tissue and the conditions of summation of the potentials of the individual cells included in the electrical circuit, for it is within the individual cells that the processes occur. Two investigators have observed this elementary condition. Glass (1933) found the normal apico-basal positivity of *Elodea* leaves to be increased by illumination of apical or middle regions, but decreased or reversed by illumination of basal regions. His results are consistent with the conclusion that the normal electrical polarity of the leaf cells is increased by illumination, save for the experiment illustrated by his figure 5, wherein a basal region originally positive toward the apex showed inverted polarity upon illumination. If it is assumed that the polarity of this region at the time of illumination was opposite to that apparently observed shortly before in darkness (possibly owing to incomplete recovery from mechanical stimulation), this experiment falls into line with the others. Essentially the same results were obtained by Brown (1938) on the apical internode of *Chara,* although the distribution of potential along this internode was not determined. The distribution may be inferred from the effects of local illumination of different regions of the internode. The inference is consistent with his demonstration that illumination of the entire plant and illumination of the apex only yield results in harmony with the distribution of potential over the whole plant by internodes.

Glass, Bose, and others reported effects of varying the intensity of incident light, but no systematic relation between intensity and E.M.F. was demonstrated. Brown (1938) has presented data from which it can be shown that the change in E.M.F. with light intensity follows a sigmoid curve over the range from 486 to 36,500 meter-candles. Unfortunately, owing to the time intervals involved, the E.M.F.'s recorded are not steady state values. These results will be considered in more detail in the discussion. Sheard (1929) stated that, in general, the response, R (= change in potential difference upon illumination), of a leaf was related to the stimulus, S (= kIt, where I is intensity of irradiation, t the time in minutes), by the equation $R = c \log S$. Visible light had little effect, infrared and ultraviolet light produced large changes in potential. No data were presented. The relative influence of I and t was not discussed, nor were their ranges of magnitude stated, although this would seem desirable. It is scarcely to be credited that intensity and time could be interchanged freely. The equation implies that a small intensity could produce an indefinitely large change in potential through being applied for a long time period. Bose (1907) believed the electrical changes produced in green plants by light to be fundamentally the same as those produced by other stimulus forms.

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[1] Aided by a grant from the Rockefeller Foundation for work in cellular physiology.

The present paper reports the effect of visible light at (at least moderately well) known intensities upon the inherent E.M.F. of the coenocytic alga, *Valonia ventricosa*. The potential difference is confined to the protoplasmic layer, 5 to 8 microns thick, whose cytological constitution has been described (Doyle, 1935). In an impaled cell complications due to summation of potentials are reduced to a minimum. The morphological simplicity of *Valonia* and the definiteness of the electrical circuit with respect to cell structures offer a maximum of advantages for the interpretation of the electrical changes in terms of cell processes. (The question whether a coenocyte may be called a cell does not appeal to the writer as constituting a real problem.) In later papers it will be demonstrated clearly that the effect of light upon the E.M.F. occurs by virtue of its absorption by chlorophyll.

Materials and Methods

The *Valonia* were collected from shallow water on the protected side of Bush Key reef. They were kept in low aquarium dishes in the laboratory, and the sea water was changed daily. They were impaled on fine glass capillaries filled with artificial sap (Osterhout, 1931) and immersed in sea water in 250-cc. pyrex beakers. Glass rods ending in a ring supported the cells from below (Blinks, 1930–1931). The sea water about the impaled cells and the artificial sap in the capillaries were changed frequently. During the course of an experiment the sea water was unstirred, save for the small circulation due to temperature convection.

Ag:AgCl electrodes made contact with the sea water and the artificial sap in the capillaries. When a liquid junction was made between the sea water and the sap the electrodes showed a difference of potential of 1–2 millivolts, the sea water being positive. Illumination of the electrodes separately or together when so connected produced no change in potential difference over the range of light intensities used. Potentials were measured with a Compton quadrant electromotor having a period of 15 seconds and sensitivities of from 2.5 to 5 millimeters per millivolt. In the data given below the E.M.F. expresses the electrical positivity of the inner (vacuolar) side of the protoplasmic layer, this being the normal orientation.

The experiments were performed in a photographic darkroom with the cells shielded from stray reflection by a black box open toward the source of light and provided with a light-tight shutter. The light source was a 400-watt Mazda spotlight with a total output (manufacturer's rating) of 8000 lumens at 115 volts. Current was supplied from a D.C. generator operated by a gasoline motor. A D.C. voltmeter was connected across the lamp during all the experiments and the speed of the motor controlled so as to maintain the voltage at or near 115 volts.[2]

Intensities were calculated from the rated output of the lamp (divided by 4 pi) and its distance from the cells, assuming the inverse-square law to hold. No measurements of absolute intensity were made. The light was shifted by hand to predetermined positions, the operation requiring 3 to 5 seconds. In order to extend the effective range of the source, as well as to reduce the heat absorbed in the box, two plane mirrors were set at the rear of the box at such an angle that the light was reflected onto the cells at all positions of the lamp. This provided approximately uniform illumination from three directions. Ninety per cent reflectance was assumed for the mirrors. The large number of geometrical calculations involved in determining the precise distances traveled by the reflected beams for different positions of the source were avoided by assuming a constant increase in the reflected over the direct path. The error introduced by this procedure is negligible at intensities less than 1200 foot-candles and rises to 2 per cent at about 1500 foot-candles. There is also a small error due to radiation losses by reflection from the surfaces of the containing vessel and the *Valonia,* for which no means of measurement or estimation were available. In consequence the stated intensities are at best close approximations.[3]

No attempt was made to maintain constant temperature in the sea water bathing the cells. A record of the temperature was kept and an electric fan was utilized to hold the temperature well below the thermal death point (about 40° C., Marsh, 1936*a*). In the results presented below, the effect of temperature has been ignored. A preliminary attempt (Marsh, 1936*b*) to correct the observed E.M.F. by means of the Q_{10}'s established for weak light or darkness (Marsh, 1936*a*) was unjustified. The corrections actually had little effect upon the shapes of the curves shown in figure 2 below. The height of the maximum was reduced and the maximum sharpened, but its position on the intensity scale

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[2] The writer is indebted to the entire staff of the Tortugas Laboratory for able assistance and cooperation. Special acknowledgment is made to Captain John Mills and Engineer Ernest Mills for their cheerful and continuous labors in holding constant the speed of the motors under exceptionally trying conditions.

[3] The intensities stated in two preliminary reports (Marsh, 1936*b,* 1937*b*) are too large by a factor of 4.2 because of an error in the assumptions concerning the source.

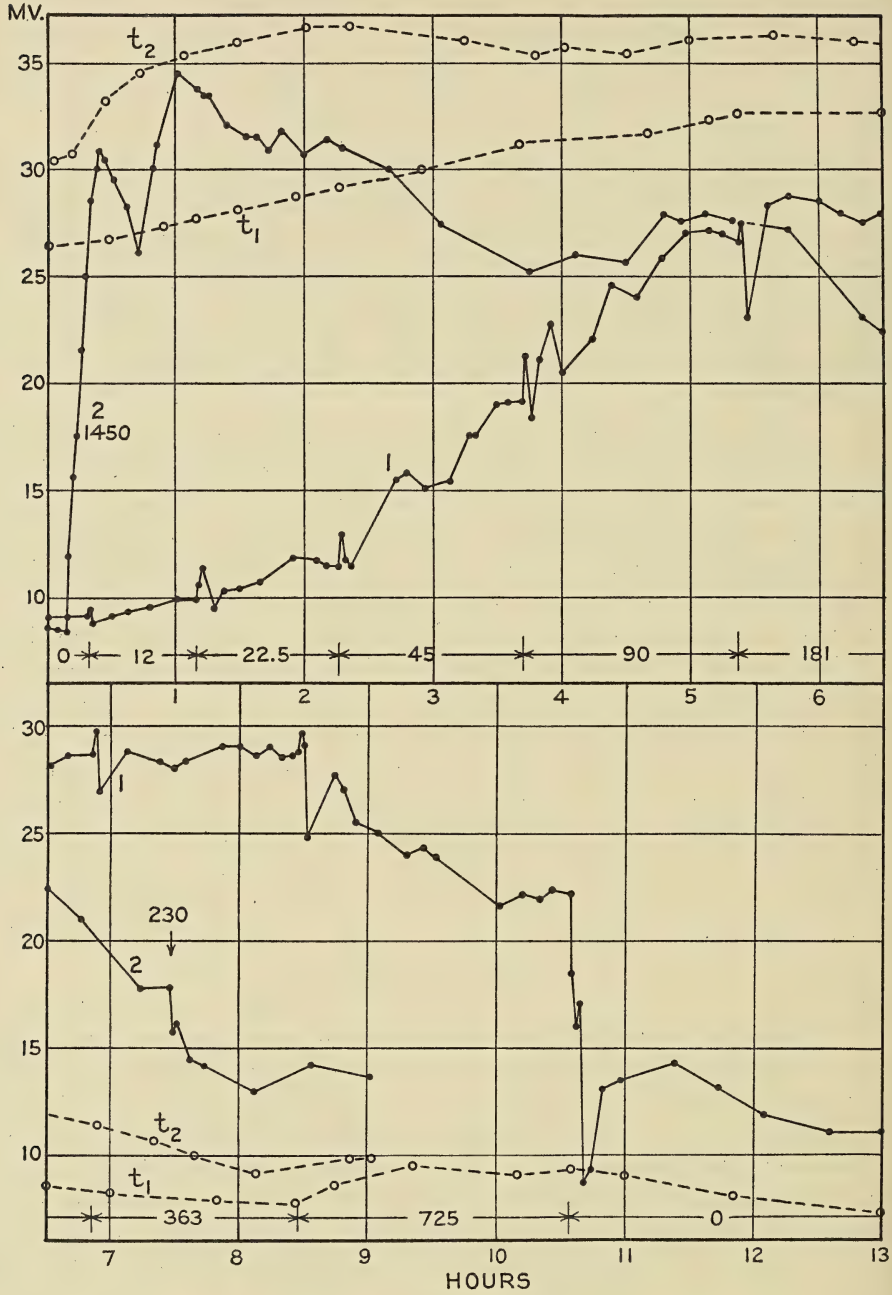

FIG. 1. E.M.F. : time record of two *Valonia* cells. Intensities in foot-candles indicated above the base line for no. 1. No. 2 illuminated at 1450 foot-candles beginning at 10 minutes and continuing to 7.5 hours, where the intensity was reduced to 230 foot-candles. t_1 and t_2 give the respective temperatures of the surrounding sea water. Temperatures in degrees Centigrade may be read on the millivolt scale in the upper half of the figure. In the lower half the base line is 30°, the 10-millivolt line 34°.

was unchanged. Subsequent experiments have indicated that in the region of the maximum the temperature coefficient is of the order of 1 (Marsh, 1937*b*), while at very high intensities it again becomes of the same order as for weak light.

Relation of the Inherent E.M.F. to Light Intensity

The time course of change of potential in a *Valonia* cell during exposure to a series of light intensities in a typical experiment is shown in figure 1, curve 1. The cell was placed in the box and allowed to remain in complete darkness until the E.M.F. had become constant at 9 millivolts. The shutter was removed from the box and the cell exposed to light of 12 foot-candles intensity. The potential rose and fell through a small initial spike, passed slightly below the value of the dark potential, then slowly climbed to a steady level at 10 millivolts. The light was then shifted to a position corresponding to 22.5 foot-candles intensity and a similar cycle of changes followed. This was repeated for the light intensities indicated above the base line, the intensity being approximately doubled at each change. The spikes became more prominent and the steady level of potential rose progressively. The shift from 181 to 363 foot-candles produced only a 0.4-millivolt change in the steady level of the potential, while the shift from 363 to 725 foot-candles caused a drop of 6.6 millivolts. The light was then excluded by means of the shutter and the potential fell through the characteristic curve recorded at the end. The curve t_1 is the temperature record of the sea water surrounding the cell. While the temperature rose steadily for the first 5.5 hours, there is no real correspondence between the temperature level and the E.M.F.

The time required for a steady level of potential to be attained varies with the magnitude of the potential change produced by the increased intensity, as will be shown below. When, however, the steady level is reached it is maintained with insignificant variations for a number of hours. An illustration is given in figure 4, curve 2.

When the steady values of E.M.F. from figure 1, curve 1, are plotted against the logarithm of the light intensity, curve 1 of figure 2 is obtained. The E.M.F. rises from the dark potential along a sigmoid curve, passes through a flat maximum at 363 foot-candles, and begins a rapid fall roughly symmetrical with the ascending limb. In figure 2 the zero line represents the dark potential level for each cell. A more typical shape is that shown by curve 4. Here the E.M.F. did not begin to rise rapidly until the intensity had reached about 100 foot-candles and the change in slope about the maximum was abrupt. The range of intensities was greater than in curve 1, so that the similarity of the ascending and descending limbs is more obvious. At 970 foot-candles the potential had fallen to the value for the steady level at the lowest intensity used (12 foot-candles). Curve 5 has a similar shape, save that the lowest intensity applied was 160 foot-candles. At 970 foot-candles the potential had a steady value of 2.2 millivolts. At 1450 foot-candles, not shown in the figure, the potential was

1.9 millivolts. In no experiment was the E.M.F. at the highest intensity reduced to the dark potential level.

Eight sets of data were obtained wherein the steady E.M.F. reached a maximum and subsequently decreased as the light intensity increased; the five not shown in figure 2 yielded curves of the general form of curve 4. The range of values was from 175 to 435 foot-candles; six of the values lay between 175 and 282 foot-candles. The mean of the eight maxima was 252 ± 19 foot-candles. The principal cause of the wide range of values found was probably the size of the intensity intervals employed in a single experiment. Since from 1 to 3 hours might be required for the potential to reach a steady level at a given light intensity, it was not possible to cover the full intensity range in more than eight to ten steps. In the majority of the experiments the intensity was approximately doubled at each step. In consequence, the highest observed E.M.F. is not necessarily the true maximum. Curve 5 of figure 2 crosses the region of the maximum with the smallest intensity intervals of any experiment, and is probably the most closely determined single value. The first three intensity points are 160, 205, and 250 foot-candles, the maximum being at the middle figure. Because of the

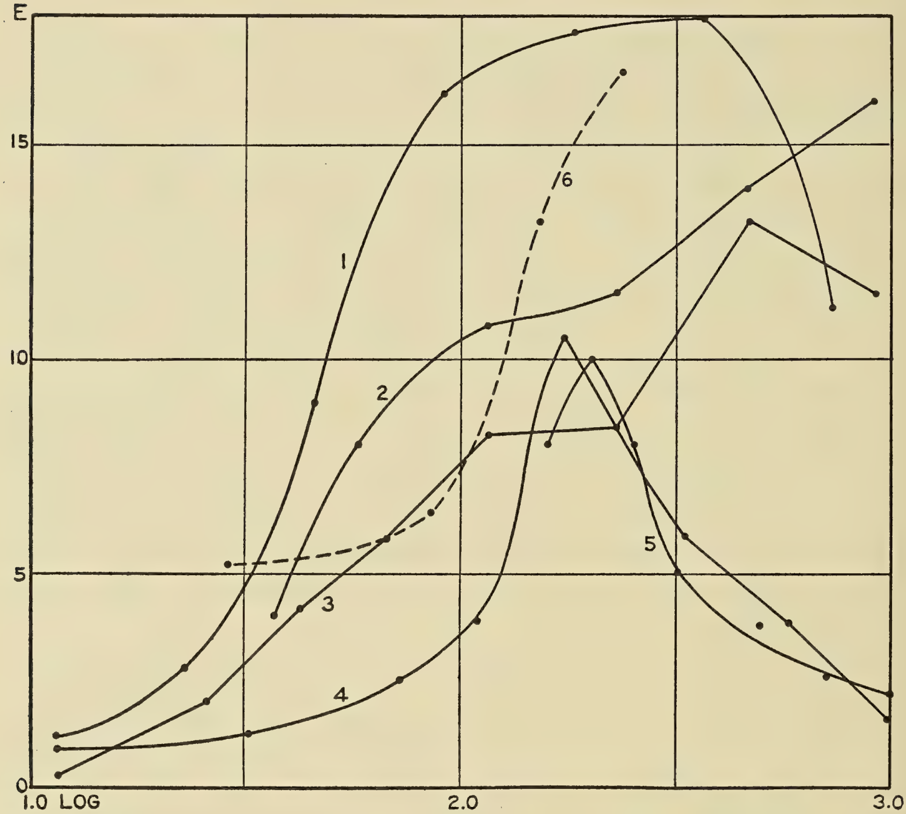

Fig. 2. Increase in steady level of E.M.F. above the dark potential for six *Valonia* cells, plotted against the logarithm of the light intensity in foot-candles.

uncertainty due to the method of determination and because it is to be expected that the position of the maximum varies from cell to cell, the mean value of about 250 foot-candles is considered only to represent the locus of a range of intensities. A larger number of determinations with smaller intensity intervals would be necessary to make the figure precise.

Three E.M.F. : log *I* curves were obtained from experiments which were similar to the above, but wherein the highest intensities applied were respectively 215, 225, and 240 foot-candles. The maxima were apparently at or near these intensity values, for the form of the curves is similar to that of the rising limb of curve 4, figure 2. One of the three is plotted as curve 6 of figure 2.

The general position of the maximum is also supported by the results of four experiments in which the intensity was reduced progressively from an initial value of about 1000 foot-candles. Two of the curves obtained were similar to curve 2 of figure 2. The E.M.F. decreased steadily with intensity; no maximum appeared, but there was a definite inflection in the curves in the neighborhood of 200 foot-candles. The other two experiments are illustrated by curve 3 of figure 2. The first reduction in intensity was followed by an increase in E.M.F.; however, the inflection appeared at the lower intensities in the same region as for the two curves described above (curve 2). The inflections are believed to mark the region of the maximum. The failure of these curves to describe a descending limb at high intensities was due to the extreme length of time required for the potential to reach a steady level under the conditions of the experiment. (See the discussion of curve 2, figure 1, in the following section.) The steady level was probably not attained at any intensity above 200 foot-candles. The exposure times at the initial high intensity were 1.25 and 1.85 hours for the two experiments illustrated by curve 2, figure 2, and 2.5 and 4.0 hours for the experiments illustrated by curve 3. While in the latter two cases the E.M.F. more nearly approached the steady level, even 4 hours is inadequate time for it to be attained under these conditions, as will appear below.

The rise in E.M.F. accompanying the first reduction of intensity in the two experiments described by curve 3, figure 2, may be interpreted as demonstrating qualitative reversibility of the decline of E.M.F. at high intensities. Two direct tests of reversibility were made with cells subjected to a series of increasing intensities whose potentials had been reduced to near the dark level at the highest intensity. The results were similar, and are illustrated by figure 3, which shows the time record of the descending limb of curve 5, figure 2. The E.M.F. decreased steadily with increasing intensity as indicated above the base line. After a short exposure to 1450 foot-candles the potential became constant at 12.7 millivolts. The intensity was then reduced to 225 foot-candles and the potential rose to become steady at 16 millivolts. In both cases the steady level at the lower intensity at the end of the series was less than the steady level at (approximately) the same intensity obtained earlier in the experiment. In one of the experiments using intermittent light, to be described later, the intensity was altered in such a way as to permit observations upon reversibility of the high-intensity effect. An increase in intensity of continuous illumination from 150 to 300 foot-candles produced a decrease in the steady potential from 34.3 to 29 millivolts. After a period of intermittent illumination at 300 foot-candles the intensity was changed back to 150 foot-candles continuous illumination. In 1 hour and 40 minutes the potential rose to 33.9 millivolts, producing almost an exact reversal of the effect of the higher light intensity. At this time the cell had been illuminated at intensities near the maximum for nearly 8 hours. The cell was then exposed to 600 foot-candles continuous illumination for 3 hours and 10 minutes, at the end of which time the potential had declined to 23 millivolts. Following a period of 1 hour in intermittent light at 600 foot-candles, the intensity was shifted back to 300 foot-candles continuous illumination. The potential rose to 19.5 millivolts during the course of 1 hour and 20 minutes. The failure of the potential to return to the same value at the higher intensity was apparently due to the long exposure at intensities above the maximum; this agrees with results described in the next section. These experiments demonstrate that the decrease in E.M.F. is a reversible photochemical event determined by the nature of the electromotive system in *Valonia,* and not due to progressive injury. It was not possible, owing to the time element, to ascend and descend the full intensity range in a single experiment, and, as will appear below, the experiment cannot be performed by beginning at the high-intensity end.

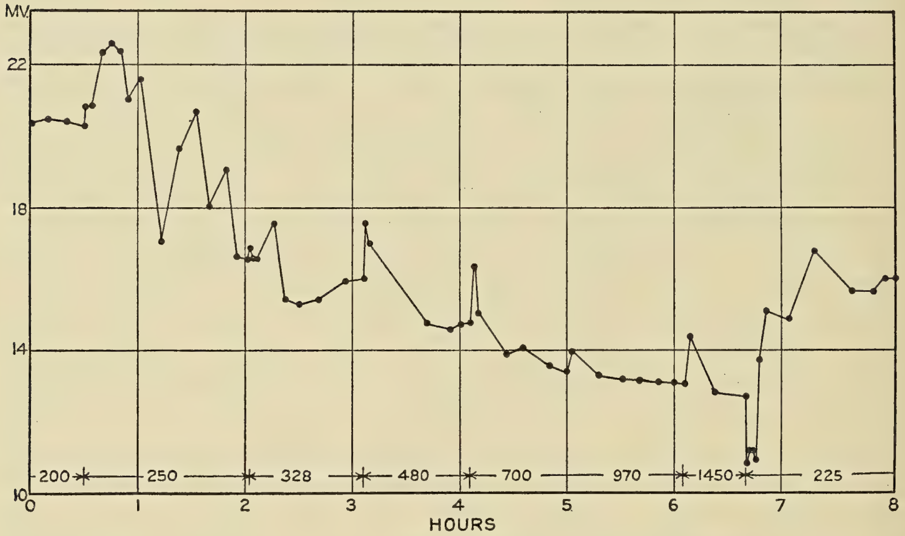

FIG. 3. Decline of E.M.F. with increasing intensity beyond the maximum. At end of record, reduction of intensity accompanied by increase of E.M.F. Intensity in foot-candles shown above the base line.

Approximately 10 foot-candles had to be applied to produce a clearly measurable increase in the steady level of E.M.F. above the dark potential. This intensity is, of course, not a threshold, for the form of the curve of E.M.F. *vs.* log intensity indicates an asymptotic approach to the dark potential at low intensities.

The value is limited by the period and sensitivity of the measuring instrument and by the conditions of the experiment. A visible spike is produced by intensities of the order of 2.5 foot-candles, but the steady-level change is of the order of 0.1 to 0.3 millivolt, and is usually masked by the slow drift which normally characterizes the inherent potential of *Valonia*. The range of values for the dark potential of healthy cells at 30° C. after a number of hours in the dark (e. g., over night) is from 6 to 12 millivolts. The values of the maxima observed in the above experiments ranged from 17.5 to 38 millivolts. The maximum was from 2 to 6 times the dark potential with an average of about 3 times. No correlation seems to exist between the dark-potential level and the maximum potential observed in light.

There was no evidence in any of the experiments that light exerted an injurious effect. Cells subjected to a series of increasing intensities showed no observable changes in color, turgor, or opacity. Their viability and their subsequent electrical behavior were indistinguishable from that of cells kept in diffuse daylight in the laboratory. The same result was found for cells illuminated for 12 hours or more at intensities of the order of 1450 foot-candles, e. g., the cell from which curve 2 of figure 1 was obtained. In experiments with carbon dioxide, to be reported in a later paper, intensities as great as 2350 foot-candles were employed without injurious effect. This is to be expected, for *Valonia ventricosa* commonly is found growing luxuriantly in positions exposed to full sun, covered at low tide by only a few centimeters, or even millimeters, of sea water. (See Steward and Martin, 1937, pp. 96 ff., for a description of the habitat at Tortugas.) Evidently precautions which have been taken by several investigators to "protect" *Valonia* from direct light are unnecessary.

The large quantity of heat emitted by the lamp and absorbed in the experimental containing vessels permitted observations on the temperature tolerance of *Valonia,* extending those previously noted (Marsh, 1936*a,* p. 9). Impaled cells on several occasions survived exposure to 37° C. for 5 to 8 hours. Two cells showed no evidence of injury on exposure to 38° C. for 5 and 6 hours respectively. In three experiments the temperature rose to betwen 39 and 40° C. for periods of 10 to 20 minutes without observable effect. In one direct test the temperature was allowed to rise unchecked. As it passed above 40° (at the rate of about 1° in 7 minutes) the potential dropped rapidly to zero. Examination showed typical heat coagulation.

The Transition Curves between Steady Potential Levels

The time curves of transition of the E.M.F. from one steady level to another when the intensity is altered possess features of interest. Their form is determined by the direction and extent of change of intensity, and by the magnitude of the difference between the steady levels of E.M.F., i. e., by the segment of the E.M.F. : intensity curve traversed. When the cell is exposed to light following a period of darkness, the E.M.F. first rises, then falls, then rises again, producing a positive and a negative spike followed by an oscillatory approach toward a steady level, as in curve 2 of figure 4. Occasionally, when the applied intensity is small, the negative spike may pass below the level of the dark potential, as in curve 1 of figure 1. When the intensity is of the order of 2.5 foot-candles the positive spike is often the only identifiable response. The two curves mentioned above with curve 2, figure 1, demonstrate that the magnitude and duration of the spikes increase with the applied intensity. A similar relation may be seen for the number and amplitude of the oscillations following the spikes and for the time required to attain a steady potential level.

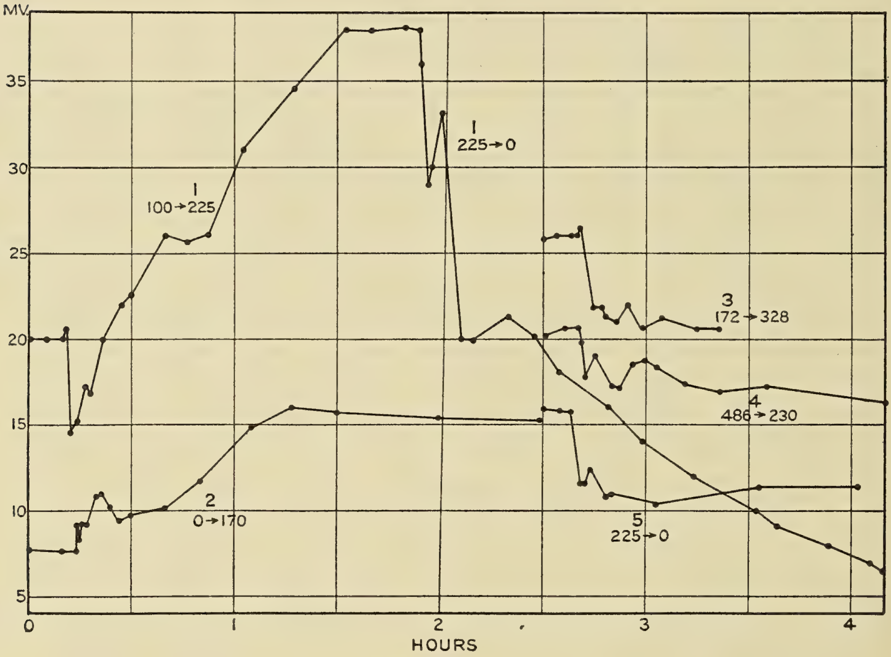

Fig. 4. Transition curves of E.M.F. between steady levels with change in light intensity. Intensity change in foot-candles indicated beneath numbers designating separate curves.

In the experiment illustrated by curve 2, figure 1, an intensity of 1450 foot-candles was applied to a cell which had been kept in the dark until the E.M.F. became constant. In the rise following the negative spike the potential reached 34.5 millivolts. The subsequent oscillatory decline was followed for 7.5 hours, at which time the potential had fallen to 17.7 millivolts and probably had not yet reached a steady level. If the potential is plotted against the logarithm of the time for this experiment, a curve is obtained roughly similar in shape to curve 1 of figure 2; the potential describes in time the same general course followed by the steady level with increasing intensity.

The time course of the potential of eight cells was followed in this manner at high intensities (1400 to 2400 foot-candles) for periods of 7 to 13 hours. Marked individual differences were observed. The potential of one cell exposed to 2100 foot-candles rose to 21.8 millivolts in about 3 hours and was maintained near this figure for an additional 7 hours. The other seven experiments yielded curves similar to curve 2 of figure 1. In only one instance did the potential decline to near the dark level. The cell was illuminated at 1420 foot-candles, reached a high point of 31 millivolts in 1 hour and 45 minutes, then followed a variable downward course which in 10 hours carried it to 11 millivolts, 2 millivolts above the initial dark level.

A test of the reversibility of the decline of the potential with time at high intensity is shown at the end of curve 2, figure 1, where the intensity was reduced from 1450 to 230 foot-candles. The potential fell through a typical intensity-reduction curve from 17.7 to between 13 and 14 millivolts. This is to be contrasted with figure 3. Other results of varying the light intensity following prolonged exposure to high intensity agree with this one in showing that the electromotive state under these conditions differs from that obtaining in a cell whose potential has undergone a decrease at high intensity at the end of a series of exposures to increasing intensities. In the former case the behavior is that of a system in which a photosensitive reserve is gradually exhausted. Evidence will be presented in a later paper to show that the reserve is CO_2.

The behavior of the potential with time at high intensities explains the form of the potential : log intensity curves begun at the high end of the intensity range (figure 2, curves 2 and 3). The value recorded for the steady level at the highest intensity in such an experiment would be taken from the region of slow decline following the spikes. This would also be true at intermediate intensities until the region of the maximum was reached. For intensities of 250 foot-candles or below, the steady level is attained in less than 2 hours, and may be maintained for as long as 6 hours.

The characteristics of the transition curves of E.M.F. with increased light intensity after a previous exposure to light may be seen in curve 1 of figure 1, and in curves 1 and 3 of figure 4. The negative spike becomes more prominent the greater the previous intensity, although the positive spike is still present. The time required to reach a steady level and the number and magnitude of the oscillations of the E.M.F. are greater the larger the E.M.F. interval produced; they are obviously dependent more on the form of the E.M.F. : log I curve than on the absolute change in light intensity.

When the intensity is reduced to zero an equally characteristic potential : time curve appears, which in general form, though not in detail, is the inverse of that obtained in passing from darkness to light (curve 1, figure 1; curves 1 and 5, figure 4). After a negative and a positive spike the E.M.F. approaches the dark level in a series of waves. The time required to reach the dark level increases with the magnitude of the E.M.F. in light. Similar curves are obtained when the intensity is reduced from a higher to a lower value (curve 4, figure 4). It will be noted that the transition curve 3 of figure 4, where an increase in intensity produced a lower steady level of E.M.F., has the same form, save for the small upward spike; the same is true for curve 1 of figure 1 when the intensity was increased from 363 to 725 foot-candles. When a decrease in intensity was followed by an increased steady level of potential (figure 3), the initial change was in the negative direction.

INTERMITTENT LIGHT

So far as could be detected in the above experiments, the change in E.M.F. occurred instantly when the light intensity was altered; if there was a latent period it was of the order of a fraction of a second. To test this point within the unsatisfactory limits of the electrometer, and to observe what changes might occur in the form of the transition curves of potential between light and darkness, a number of experiments were performed using intermittent light of varying frequency. It was also thought to be of interest to discover whether intermittent light would be more effective than continuous light in determining the value of the potential in *Valonia,* as it has been shown to be in other material for the photosynthetic rate.

Warburg (1919–1920) found the Bunsen-Roscoe law to hold for intermittent light of low intensity, but not for intensities two or more times that necessary to produce the maximum photosynthetic rate. Using equal light-dark intervals, he found CO_2 assimilation in *Chlorella* to be greater with intermittent light than with continuous light of the same intensity and the same total radiant energy. The increase was greater the higher the frequency of light-dark periods: at 4 per minute the increase was 14 per cent, at 400 per minute 56 per cent, and at 16,000 per minute 88 to 96 per cent. Emerson and Arnold (1931–1932) obtained an increase of from 300 to 400 per cent at 3000 flashes per minute by lengthening the dark period between flashes.

Figure 5 shows the time record of the potential of a cell exposed to intermittent light at 170 foot-candles intensity. The dark periods are indicated by the shaded rectangles along the base line. Darkening was produced by a shutter operated by hand.[4] In the upper half of the figure a dark period and a light period of 30 minutes produced typical transition curves. Equal light and dark periods of 10, 5, 2.5, 1, and 0.5 minutes were then tested, each series separated by an interval in continuous light. It will be seen that the transition curves are essentially unchanged in form and but little changed in magnitude. The curves in light or in dark are simply deleted from behind; the potential traces out that segment of the beginning of the curve which occupies a time interval equal to the light or dark period. The mid-point of the short oscillations at the last three frequencies follows roughly the same course as the first potential curve in the dark at the beginning of the figure. This feature was still more marked at frequencies of 4 and of 12 per minute, not shown in the figure. At the latter frequency the amplitude of the oscillations was about 0.7 millivolt; the duration of the light or dark period was one-third the period of the electrometer. (In another experiment with a 1-second period the oscillation of the potential was still visible, although of uncertain magnitude.) After 30 minutes' exposure to intermittent light at 12 light-dark periods per minute, the cell was exposed to continuous light at 85 foot-candles intensity. The potential was 0.8 millivolt higher than the high point of the oscillations with intermittent light. Other attempts at this intensity gave the same result.

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[4] The writer gratefully acknowledges the assistance of Mr. George Streeter in manipulating the shutter.

In later experiments a rotating sector disk giving 2 equal light-dark periods per revolution was used to interrupt the light. The disk was turned by a small electric motor whose speed was such as to give 8 to 10 light-dark periods per second. With this device the effect of intermittent light was compared with that of continuous light of half the intensity over a range of intensities from 30 to 1500 foot-candles. Six *Valonia* were tested. In four experiments there was no observable difference between the potential in intermittent light and that in continuous light of half the intensity. A fifth experiment yielded a doubtful result because of the sequence of intensities employed. A sixth experiment gave a distinct difference. A cell previously in the dark was exposed serially to 100, 150, and 300 foot-candles continuous light. The second intensity change produced a decrease in potential from 34.3 to 29 millivolts. Intermittent light at 300 foot-candles was then applied; the potential again decreased, to become steady at 25.3 millivolts. The cell was then reexposed to 150 foot-candles continuous light. The potential increased, to become steady at 33.9 millivolts. Next

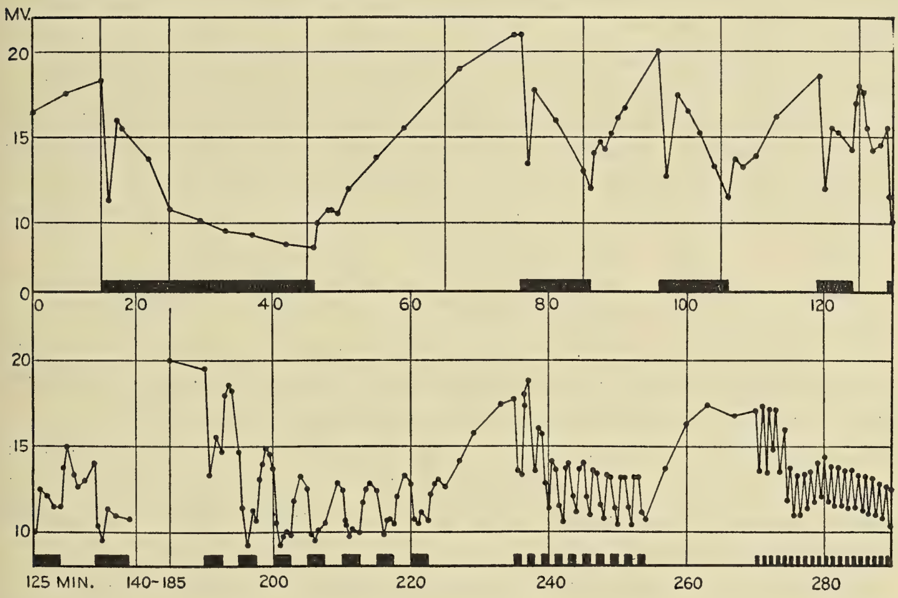

FIG. 5. Time course of E.M.F. with alternating periods of light and darkness. Dark periods indicated by shaded rectangles on base line. Length of dark periods, serially: 30, 10, 5, 2.5, 1, and 0.5 minutes.

600 foot-candles continuous light was applied; the potential fell to 23 millivolts. Intermittent light at 600 foot-candles produced a further fall to 16.7 millivolts. When the intensity was changed back to 300 foot-candles continuous light, the potential rose to 19.5 millivolts, at which point the experiment was discontinued. The effect of intermittent light in both instances resembled the effect of continuous light of an increased intensity; continuous light of half the intensity, which would possess an equal radiant energy content for a given time interval, produced an opposite effect upon the potential. This is in qualitative agreement with Warburg's results.

The fact that only one experiment out of six yielded clear-cut evidence of increased photosynthetic rate with intermittent light does not necessarily detract from the value of that experiment. Both light intensity and available carbon dioxide determine whether the difference between intermittent and continuous light will appear. No data have been published on photosynthetic rate at different light intensities in *Valonia,* so that saturation intensities are unknown.

Discussion

The electrical polarity of *Valonia* is, as indicated by the results of the experiments described above, intimately dependent upon the intensity of incident light, a relation which has been uniformly ignored in the earlier literature. A similar relation presumably holds for other green algae. It will be shown in later papers that the effects upon the E.M.F. of a variety of chemical and physical factors in the environment show characteristic differences in light and in the dark (Marsh, 1938).

The steady potential in light is dependent upon light intensity alone and is independent of the time of application of illumination; it thus partakes of the nature of an equilibrium. The transition E.M.F. between steady levels is more variable, being dependent upon time, the magnitude and direction of change of the steady-level interval, previous illumination, etc. It might be pointed out that even the transition E.M.F. is not determined by the intensity-time product. It seems probable that the failure of earlier workers to distinguish between these two aspects of the electrical effect of light has been in part responsible for the diversity of viewpoints. In *Valonia* neither aspect can be advantageously interpreted in terms of a stimulus-response mechanism.

To account for the large number of facts connecting continuously maintained bioelectric potentials and cell respiration, Lund (1929, 1931) formulated the theory that the potential arises from an oxidation-reduction system, wherein the electromotively active materials are produced in the normal respiratory processes and maintained at flux equilibrium concentrations. The electrical polarity of a cell was assumed to be the algebraic sum of the oppositely oriented potentials manifested at at least two loci within the cell (phase boundaries or plasma membranes acting as electrodes). Upon the basis of Lund's formulation the writer has shown (Marsh, 1935, 1937*a*) that a quantitative relation may be developed between the E.M.F. and the kinetics of the respiratory process. The polarity potential of a cell is expressed as

$$E_p = -\frac{RT}{nF}\, ln\, \frac{P_{c_1}}{P_{c_2}} = -\frac{RT}{nF}\, ln\, r'$$

where P_c represents the flux equilibrium oxygen tension at a locus and the subscript 1 designates the locus which is positive in measuring circuit. P_c may be expressed as a function of the oxygen pressure of the medium, P, and the velocity constants of the respiratory reactions.

When r' is plotted against P (Marsh, 1935, p. 687), a curve is obtained which rises from 0 at $P = 0$, passes through a maximum, and descends to 0 as P becomes indefinitely large. The descending limb has the lesser slope. When the E.M.F. is plotted against log P, the general characteristics of the curve are unaltered; the descending limb is shortened and the curve becomes more symmetrical. The similarity of these curves to the E.M.F.: log I plots in figure 2 of this paper is striking, and suggests similarity of origin. The temperature coefficients of the E.M.F. in light indicate that the saturating light intensity for photosynthetic rate lies somewhere beyond the intensity producing the maximum E.M.F. It is reasonable to assume that over the range of intensities employed, photosynthetic rate, and, consequently, oxygen pressure, is proportional to light intensity, so that log P could be substituted for log I. The same conclusion may be drawn from the reversibility of the decline at high intensities, which cannot be explained by assuming the photosynthetic rate to reach a constant level at some intermediate intensity. Reversibility of the decline at high intensities is itself evidence for the existence of the flux-equilibrium relation. Reversibility would not be found if the decline were due to solarization or other injury, nor could it be accounted for by depletion of the carbon dioxide supply. The behavior of the inherent E.M.F. of *Valonia* with light intensity fulfills the predictions for an oxidation-reduction system at flux equilibrium, where oxygen pressure, produced through photosynthesis, is the immediate determining factor. It will appear from work to be reported later that a complete description of the relation of the E.M.F. to light is somewhat more complex than is here indicated.

From his study of the effect of light on the electrical polarity of *Chara* internodes, Brown (1938) also concluded that oxygen was the primary factor in determining the potential. With the internode exposed to a gaseous atmosphere he found the effect of illumination at a given intensity to decrease as the oxygen pressure of the atmosphere was raised, and showed that this effect might be explained on the basis of the flux-equilibrium concept. Illumination of a plant previously in darkness produced a rise in potential which passed through a high point in about 10 minutes and descended to a steady level in 20 to 40 minutes. Brown did not determine the relation of the steady level to light intensity. He subjected internodes to alternate 10-minute periods of light and darkness, using intensities from 486 to 36,500 meter-candles. If the E.M.F. obtained at the end of the period of illumination is plotted against the logarithm of the intensity, a sigmoid curve is obtained which is similar to the rising limb of curve 1, figure 2 of this paper. If the relation between E.M.F. and intensity in *Valonia* had been obtained in this way, the high points of the transition curves of E.M.F. would have increased over the whole intensity range and no maximum would have been obtained.

The specification of oxygen pressure as the immediate cause of the change in E.M.F. with light intensity raises an interesting question. Blinks, Darsie, and Skow (1938) found the E.M.F. in *Halicystis* in darkness or weak light to be independent of oxygen tension from 100 per cent down to 2 per cent. A similar independence has been observed by the writer for *Valonia* in the course of experiments with CO_2. The oxygen tension was not measured, but pure oxygen, air, and commercial nitrogen passed through the sea water were virtually alike in their effects upon the P.D. in darkness. The effective oxygen pressure in the protoplasmic layer due to photosynthesis lies within this range. An attractive solution to this difficulty lies in the work of Kautsky and his coworkers. Observations on the fluorescence of chlorophyll in the presence and absence of oxygen and under other conditions led Kautsky to the conclusion that a metastable, excited oxygen molecule with an energy level above that of atmospheric oxygen is produced in photosynthesis. (References to and a discussion of Kautsky's conclusions may be found in Taylor, 1935, and Mestre, 1935.) The intervention of such an excited molecule in the electromotive process would be in harmony with the results reported in this paper and would explain the apparent discrepancy arising from the observations of Blinks. Active oxygen might also account for the increased respiration of green plants upon illumination (Burkholder, 1936, pp. 39–40).

Under a given set of conditions of light intensity and previous illumination, the transition curves of E.M.F. show great constancy of form and of reproducibility. They evidently represent the electrical aspect of an electrochemical process which follows a definite time course under the influence of a material photochemically produced at a definite velocity. The time relations are such that the form of the curves cannot be due to the brief fluctuations in photosynthetic rate observed by Li (1929). The form is consistent with the expectations from the flux-equilibrium concept, which necessitates a difference in the velocity of transformation of the electromotively active materials at the two loci within the cell (Marsh, 1936*a*).

SUMMARY

1. When the intensity of incident light is altered, the inherent E.M.F. of an impaled *Valonia* cell undergoes a characteristic cycle of change with definite time relations, following which a steady level is reached.

2. The steady level of E.M.F. plotted against the logarithm of the light intensity rises from the dark potential along a sigmoid curve to a maximum at about 250 foot-candles, then descends along a similar curve toward the dark potential.

3. The decline in potential with light intensity beyond the maximum was reversible. No injury was detected at any intensity.
4. The transition curves between steady potential levels show characteristic features of form and duration which are determined by the direction and magnitude of change in light intensity and by the magnitude of separation of the steady potential levels.
5. The effect of intermittent light with equal light-dark periods was similar to that of continuous light of half the intensity, save in one experiment, wherein the effect was similar to that of continuous light of increased intensity.
6. It is concluded that the effect of light on the E.M.F. is due primarily to the release of oxygen in photosynthesis.
7. The results are in harmony with the predictions of Lund's flux-equilibrium concept for an oxidation-reduction system as the source of the electrical energy.

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PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

V

The Growth of Valonia ventricosa J. Agardh and Valonia ocellata Howe in Culture

With a Note on the Sap Composition of Valonia ocellata Howe at Tortugas

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Birkbeck College, University of London

With eight plates

[ISSUED October 31, 1939]

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The Growth of Valonia ventricosa J. Agardh and Valonia ocellata Howe in Culture

With a Note on the Sap Composition of Valonia ocellata Howe at Tortugas

Introduction

So much physiological work has been done using species of *Valonia* that their mode of development has especial interest. Individual plants cannot be kept under continuous observation during growth in the natural habitat, and the fragmentary ideas in the literature, summarized by Steward and Martin (1937), concerning the mode and rate of their development have been derived from the casual impressions of collectors and the behavior of mature vesicles during physiological experiments.

It has long been known that, granted suitable precautions, relatively large vesicles of *Valonia* would persist in aquaria for many weeks or even months. Sluggish expansion, often accompanied by a change of shape, has supplied the only evidence of "growth." No previous record exists, however, of vesicles of *Valonia ventricosa* having been grown again as normally attached plants with rhizoids after they were removed from their natural substratum in the usual manner of collection, or of the production from isolated vesicles of one of the normally branched species, e. g. *Valonia macrophysa,* of the characteristic habit of the plant in nature. Steward and Martin (1937) emphasized the fact that until *Valonia* had been successfully cultured the factors which determine its growth and development could not be investigated and that this necessarily limited the use of these organisms in the investigation of physiological problems for which their morphology seemed particularly suitable.

The Species Used

Living material of *V. ventricosa* and *V. ocellata* was collected at the Dry Tortugas, Florida, U. S. A. These species were chosen because of the difference in their morphology. *Valonia ventricosa* produces a thick-walled vesicle which may attain great size (up to 30 cc. or more) but does not form new vesicles by branching in the upper part. The conditions under which this plant grows have been described elsewhere (Steward and Martin, 1937). The published photographs show their characteristic appearance in their habitat and as they are normally collected.

By contrast, *Valonia ocellata* forms a compact, tissue-like mass, usually growing on coquina rock, or even on submerged masonry in tide-filled pools. Whereas each plant of *V. ventricosa* apparently forms but one vesicle, every mass of *V. ocellata* contains a large number of much smaller vesicles, which are of two kinds. The larger, approximately pear-shaped, vesicles rarely exceed 0.025 cc. in volume and, like the more familiar vesicles of *V. ventricosa* and *V. macrophysa,* contain within a thin cytoplasmic layer a clear aqueous sap. A representative group of such vesicles, dissected from a large mass, is shown in plate 1, figure 1. Scattered groups built up from smaller segments also occur, and these Taylor (1928) aptly termed "moruloid" masses. The moruloid masses are about the size of the larger vesicles referred to above. In the moruloid mass the small, cellular compartments are compressed together to form a compact tissue-like mass, each segment of which much be separately punctured (with a minute pipette) to extract sap. The form of these will be described with their development.

Valonia ocellata grows attached by vesicles which extend into long cylindrical processes, and these penetrate the interstices of the substratum. Plate 1, figure 2[1] shows a typical mass of *V. ocellata* which is somewhat incrusted with calcareous matter and is still attached to the coral fragment on which it grew. Typical rhizoidal processes (right and left center), large vesicles (extreme left), and moruloid masses of small vesicles (center) are all evident in the plant as it grew in nature.

Valonia in Transit

To transfer living *Valonia* from the Dry Tortugas to London presented difficulties which were overcome as follows. Only plants which had been carefully cleaned after collection and had been kept under observation in aquaria for some time were used. Quart preserving jars fitted with glass lids, rubber rings, and spring clips normally used to make a gas-tight seal were used. The jars were only half filled with fresh sea water and enough vesicles were added to cover sparsely the base of the jar. The rubber rings were omitted and in transit the glass tops were kept in place by the clips, but every opportunity was taken to loosen the lids for ventilation. These precautions were suggested by experience, which showed that *Valonia* in closed containers during experiments develops gas, and vesicles succumb if this is not removed. The bottles were protected against direct sunlight and in August and September temperature was never too low. The sea water was changed once, in mid-Atlantic with water direct from the Gulf Stream. By this means all but the largest vesicles of *V. ventricosa* reached England alive, after which they were kept in diffuse light at 20 to 24° C. until new cultures were established. In unchanged, clean cultures *Valonia* shows surprising endurance. Some vesicles in the original Gulf sea water were reserved for purposes of "seeding" new cultures, and some of these contained viable material 15 months after they were collected.

Culturing Valonia ventricosa from Aplanospores

The larger detached vesicles are capable of only very sluggish expansion, and this was completed long before any further attempts were made to raise a new actively growing culture. If the smaller vesicles (0.5 to 0.75 cm. in diameter) remain undisturbed, numerous hapteron cells develop, and these, which form between vesicles in close proximity and are just visible to the naked eye, become so closely entangled that many vesicles adhere and appear to be connected like beads on a chain. The tangled weft of thin-walled hapteron cells and the way they become inflated on contact is seen in plate 2, figure 1. The development of hapteron cells occurs most readily on the smallest vesicles, and this, as shown by experiments at Tortugas with very small graded vesicles (order of 600 to a gram fresh weight), is markedly stimulated in sea water enriched with nitrate and phosphate. Successful cultures, however, cannot be started from vesicles which are large enough to be collected by hand. A growing stock must be started from aplanospores.

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[1] Photograph from a specimen preserved in formalin–sea water.

Kopac (1933) has shown that the formation of aplanospores may be induced at will in *Valonia ventricosa* by making, with a micro-manipulation needle, a puncture so small that collapse of the vesicle does not occur. Steward and Martin (1937) noticed incipient aplanospore formation in cultures treated with ammonia. A variety of causes may produce the same effect, and in a stock of vesicles kept for long periods in unchanged sea water, vesicles which contain aplanospores can be obtained almost at any time, although there is a great variation even in the vesicles in the same container.

Occasionally, within an old vesicle, aplanospores reach a diameter of several millimeters, and such have occasionally been encountered in nature at Tortugas. However, in marked contrast to *V. ocellata,* the development of aplanospores within the old vesicle of *V. ventricosa* is arrested and they remain so minute that they are usually evident only upon microscopic examination. Plate 2, figure 2 shows a sparse population of thick-walled spores which had ceased to grow after about two months within the old vesicle. In some vesicles the sporelings remain very small and their number is very large—probably limited only by the number of nuclei the vesicle contained. Two or three such vesicles yield an inoculum from which several hundred individuals may survive.

Valonia ventricosa Aplanospores

The aplanospores, formed in response to mechanical stimulation (e. g. puncture), acquire a visible, differentiated surface layer very quickly—even as early as 3.5 hours from the stimulus to spore formation (Kopac, 1937). Globules of oil, which coalesce with fluid membranes of cells (Chambers and Kopac, 1937), do not penetrate the film which quickly surrounds the globule of protoplasm destined to become the aplanospore. The first appearance of cellulose is of no little interest. Kopac (1937) failed to identify it conclusively after some 30 hours after aplanospore formation, although at this time a solid film was present on the surface. However, Kopac (1937) used the familiar chlor-zinc iodide reagent, and tests on even the mature wall are negative with this reagent, so that there is no reason to suppose that the first-formed aplanospore wall is different from that laid down later.

The resting aplanospores have a thick anisotropic wall which may be surrounded by an isotropic mucilage sheath. This sheath,[2] coagulated into drops by spirit, can be seen on sporelings in plate 3, figure 1, in some of which division has occurred. Between crossed Nicols the appearance of a spore suspension is independent of the orientation of the specimens to the Nicols and in optical section shows, along two diameters, dark lines of total extinction which are at right angles and are parallel to the two vibration directions of the Nicols. A region of maximum intensity appears at four points on the wall on radii at approximately 45° to the planes of vibration of the Nicols. Passing from these points at which double refraction is greatest, the intensity of light transmitted fades as the objective is focused, at a higher or lower level, on the surface of the sporeling following a meridian, until total extinction is again obtained at points on the surface of the vesicle through which the incident light now passes, without double refraction, normally to the surface of the wall. Admittedly the whole spherical spore is a complex system to interpret, but it is evident that at this stage in its development the wall of the spherical sporeling of *Valonia ventricosa* behaves as if the cellulose chains were symmetrically orientated about the center of the sphere so that, viewed in imagination from this point, the molecular arrangement seen along any radius would be the same. By use of a gypsum "sensitive violet" plate inserted in a direction at 45° to the Nicols it can be seen that colors higher in Newton's series (blue and blue-green) occur along diameters parallel to the fast direction in the plate, and along diameters parallel to the slow direction lower colors (yellow) in the scale are obtained. It is evident therefore that in the sporeling the radii are always fast directions (smaller refractive index) and the tangential directions are slow (larger refractive index), which is consistent with a tangential arrangement of cellulose units in the wall. The degree of radial symmetry in the wall of the spore described above has disappeared in even small vesicles, only a few millimeters in diameter, which show more complex polarization figures. Only when viewed between crossed Nicols and along a diameter passing through the "base" where rhizoids are attached is the uniaxial figure still obtained. If the specimen is so orientated that its axis from rhizoids to tip is at right angles to the direction of the incident light, then rotation of the specimen produces a series of typical biaxial figures. Clearly after the vesicle has thickened its wall and grown as an attached plant with a definite orientation determined by the presence of rhizoids, its radial symmetry has disappeared. The evident symmetry of the young sporeling seems hardly compatible with the structure assigned to the old wall of *Valonia* by Preston and Astbury (1937), since they attribute to each vesicle two definite "poles." Because the first-formed aplanospore wall has been formed on a naked protoplasmic surface, its structure should be elucidated fully, and it is clear that a developmental study of the wall is necessary to determine the full details of its molecular structure. The aplanospores of *V. ventricosa* may divide, usually asymmetrically, while they are still within the old vesicle, and the cell, or cells, thus cut off elongate to form a rhizoidal process. In contrast to *V. ocellata,* it should be noted that the rhizoid is separated by a wall from the cell which later forms the vesicle. Plate 3, figure 1 shows an aplanospore with a developing rhizoid on which the mucilage sheath has aggregated into droplets. The cylindrical rhizoids and the spherical spore present a different appearance between crossed Nicols. Plate 4*A* shows a specimen photographed on the stage of the polarizing microscope with one Nicol withdrawn to show the hairs which indicate the vibration direction of the Nicols for comparison with plate 4*B* and *C*. When the length of the rhizoid lies along one vibration direction of the Nicols its double refraction disappears (pl. 4*B*), but when it is orientated at approximately 45° to this direction the weak double refraction of this thin wall attains its greatest intensity. Preston and Astbury (1937) were inclined to the belief that the rhizoid develops at one "pole" of the vesicle because of certain structure peculiarities at that point[3]—peculiarities which favored the cellulose chains being "pulled out" into a cylindrical cell. The developmental evidence is rather that the rhizoid may originate *anywhere* on the spherical surface of the spore, just as the more ephemeral hapteron cells originate over a large part of small vesicles, and therefore that the structural differences in the molecular architecture of the wall of rhizoid and vesicle laid down after this has occurred are the resultants, not the causes, of the markedly different development of rhizoid and vesicle respectively. If the X-ray evidence of Preston and Astbury (1937) obtained on a large vesicle cannot be reconciled with a sporeling or small vesicle which may throw out branches at *any point* on its surface, there still remains much to be learned about the molecular structure of the wall of *Valonia*, and special attention should be devoted to the first-formed wall on the sporeling.

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[2] The mucilage may be a factor enabling sporelings to adhere to a suitable surface before rhizoids are formed; see page 91.

Only very limited success attended many efforts to start actively growing cultures by inoculating sea water with a dense suspension of aplanospores washed out of *V. ventricosa* vesicles. It is true that many sporelings which rested on the base of the container undoubtedly grew, even increased in size by over 100 per cent, but the cultures did not persist. In a superabundance of sea water to which nitrate and phosphate were added, nutritional factors should not be limiting. The impression was unavoidable that the conditions for active growth were closely associated with the successful attachment of the vesicle to a suitable substratum. Many trials were unsuccessful; even the use of a substratum composed of a "sand" of coarse fragments of calcareous *Halimeda* scales from Tortugas failed. Not until the spores were "sown" on chipped marble did they become permanently attached.

Coarse marble chips, as sold for use in Kipp's apparatus, were washed thoroughly in running tap water, rinsed in sea water, and placed as a compact layer covering the base of a 4-l. bottle half-filled with sea water. The sea water was inoculated with a suspension of aplanospores, which were allowed to settle at random on the marble. The cultures, fitted with a loose metal cover and screened from direct sunlight, remained undisturbed for a few weeks in the greenhouse. By this time the sporelings formed a dark green felt clearly visible on the white marble, and they were transferred, still attached, to about 30 l. of sea water which about half filled a large glass tank, on the base of which the marble chips were sparsely arranged. The tank was placed in a greenhouse used for growing subtropical plants, in which the temperature was high (90 to 95° F.) and the atmosphere almost saturated with water. The screening commonly adopted in normal greenhouse practice in the summertime was supplemented by a thick sheet of ground plate glass. For over six months the cultures grew steadily in sea water to which only infrequent additions of a few parts per million of potassium nitrate and phosphate were added as required to maintain the dark green appearance of the healthy vesicles. Some trouble due to the formation of a scum (mainly of diatoms) was experienced, so that the surface of the water was periodically cleaned with filter paper. Blue-green algae tended to grow on the chalk unoccupied by *Valonia,* but their presence was not harmful to the *Valonia.*[4]

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[3] Preston and Astbury in their summary state that "the development of the rhizoid has been investigated." This is misleading, for all the observations were made on the structure of the mature vesicle; the views on development expressed are speculative.

By analogy with the development of cells of flowering plants, it might have been expected that the vesicle would pass through a period of rapid expansion till its full size was reached, followed by a period in which the wall thickened. The culture experiments described show no evidence of this, and it must be assumed that the vesicle grows and the wall thickens simultaneously.

The progress and extent of the growth which occurred can be seen in plate 5*A, B, C,* taken at 1½, 6, and 10 months respectively.[5] Filaments first arise from the aplanospores and spread on the surface of the marble, and may attain a length many times the diameter of the young vesicle. The somewhat extensive development of the filament, which in general appearance was reminiscent of *Vaucheria,* was unexpected. Plate 6*A* shows a vesicle with filaments sufficiently detached from the marble to reveal their extent, and plate 6*B* shows a larger vesicle with two well-defined rhizoids, as they appeared about 8 weeks from "sowing" on marble. At this time the vesicles were minute (see pl. 5*A*), but some 4 months later the marble bore a dense crop of closely clustered vesicles which reproduced faithfully the appearance of young plants in their natural habitat (see pl. 5*B*). A few vesicles had attained a diameter of about 0.4 cm., but the majority were smaller than this at the end of their first summer when growth subsided. Under culture conditions *Valonia ventricosa* grows best when the temperature approaches the summer temperatures of Tortugas. Osterhout has stated that *V. macrophysa* is most active at Bermuda in the winter, and he suggests that at the high summer temperatures of Tortugas it is "not healthy." Experience of growing other species of *Valonia* throughout the year does not substantiate Osterhout's conclusions; it is most active under the best summer conditions obtainable in England under glass.

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[4] On the contrary, the writer suspects, though quantitative evidence is lacking, that the unrestricted growth of the blue-green algae in certain cultures facilitated the growth of the *Valonia.* In view of the ability of certain species of blue-green algae to fix atmospheric nitrogen, this point may have some significance.

[5] After 12 months' growth the largest vesicles had a diameter of 10 mm. Vesicles at 8 mm. diameter were, however, numerous.

Once *V. ventricosa* is established on the marble, the surface becomes covered by close clusters (pl. 5*B, C*) of vesicles which, as they multiply, appear to colonize previously unoccupied surface. The close clusters recall the typical habit of the plant in nature, where vesicles are rarely solitary. To understand how these arise one must appreciate another important aspect of the growth of *V. ventricosa.*

Illustrations of *V. ventricosa* commonly emphasize the few, usually three, rather stout, thick-walled rhizoids which are the principal means of anchorage, but exclude reference to the much more numerous thin-walled filaments which develop from the crowded "lentiform" cells (Börgesen, 1913) or "Uhrglaszellen" (Oltmanns, 1922; Kuckuck, 1907). The extent of this filamentous portion of the plant cannot be appreciated from specimens as they are commonly collected, for most of it remains behind on the porous substratum. Using specimens cultured on marble, it was proved that the filaments penetrate the substratum. Small marble chips bearing numerous vesicles were treated with dilute hydrochloric acid. Long after the marble had shrunk to a mere fraction of its volume, the vesicles still remained attached by delicate strands which penetrated several millimeters into the marble and ramified over and near the surface. Börgesen (1913) recalled that Kuckuck (1907) saw small "daughter vesicles" which occasionally develop on the outgrowth of "lentiform" cells, and Börgesen himself noted that these again may develop "lentiform" cells and rhizoids. Börgesen considered it probable that these minute vesicles, borne on filamentous outgrowths from the base of a larger vesicle, could also grow into large vesicles. From the observations made on growing cultures there can be no doubt that this actually occurs and that the dense clusters formed in culture and in nature originate in this way.

Culturing Valonia ocellata

The larger, pear-shaped vesicles of *Valonia ocellata* (pl. 1, fig. 1) also form aplanospores, but, unlike those of *V. ventricosa,* these may expand inside the "parent" vesicle until they fill its entire volume. Various stages may be seen in plate 7*A* to *C*. Plate 7*A* shows the larger vesicles of *V. ocellata* containing only a few growing aplanospores, and plate 7*B* illustrates several stages in development, showing the aggregates of still spherical globules within the wall of old vesicles. Plate 7*C* shows a typical "moruloid mass," which is similar to those which may be seen in nature and were described by Taylor (1928), but which was formed by the development and mutual compression of developing aplanospores within the old vesicle, the remnant of the wall of which can be seen still attached. The moruloid masses which occur in nature doubtless develop in the same way.

According to Börgesen (1913) and also the summarized account given by Fritsch (1935), the protoplasm of *Dictyosphaeria* fragments and the multicellular sheath is formed by the expansion and mutual compression of the globular fragments thus formed. Börgesen called this process "segregative cell division" and states that it occurs in *V. utricularis* (Roth) Ag. from the Danish West Indies, which according to Taylor (1928) is synonymous with *V. ocellata* Howe as found at Tortugas. Börgesen describes and illustrates numerous lens-shaped cells particularly along the margins of the small compressed segments, and states that these develop haptera which penetrate contiguous cells and bind the whole mass. In material like that shown in plate 7*C*, the lens-shaped cells were easily recognized after clearing, but they did not occur in all the compartments of the "moruloid mass," and haptera which passed from one segment to another were not demonstrable.

Aplanospores washed out of the vesicle will slowly grow, unattached, in sea water and regenerate the characteristic pear-shaped vesicles previously illustrated (pl. 1, fig. 1; pl. 7*A*). Although growth in culture is slow, in a few months vesicles as large as ever occur in nature in this species can be grown. However, both unsegmented vesicles and the compact masses of compressed "cells" described above may throw out cylindrical processes which, growing at the tip, may attain a length many times that of the pear-shaped vesicle from which they emerge. Unlike the rhizoid or minute hapteron cells of *V. ventricosa,* these are not cut off by walls. By unilateral illumination with a horizontal beam of light from a 500-watt lamp with a reflector, almost every vesicle or sporeling was stimulated to develop a cylindrical rhizoidal process on the side away from the light, and, after they emerged, these structures continued to grow *at their tip* for several weeks in a direction away from the light and along the surface of the dish. The general appearance of these rhizoids and a single group of sporelings with positively geotropic and negatively phototropic rhizoids is shown in plate 8, figure 1*A, B.* The rhizoidal processes branch though without septa, and, when vesicles are loosely attached to floating matter so that their rhizoids grow downward and away from the light into a deep tank of sea water, *V. ocellata* may assume the striking form illustrated in plate 8, figure 2. In this form the habit of *V. ocellata* is reminiscent of the appearance of species of *Botrydium,* familiar from illustrations in textbooks (Fritsch, 1935), in which a branched rhizoid grows into soft mud. *V. ocellata* is clearly able to produce a filament with apical growth and dichotomous branching, a characteristic not usually attributed to *Valonia,* although it is a familiar feature of other members of the Siphonales. The best growth of the cylindrical filaments was obtained when supplementary artificial light was used. It is possible that the red rays, in which this source is rich, stimulated the development of the long filaments in a manner somewhat similar to that observed by Charlton (1938) for spores or growing cells of the prothallium of ferns.

In *V. ocellata* spherical vesicles and cylindrical filaments can be examined on the same "cell." These show a different appearance between crossed Nicols. Specimens as illustrated in plate 8, figure 1*B* when examined in this way show that complete extinction along the filament is obtained when its length lies in the direction of vibration, whereas the spherical portion exhibits biaxial figures like those of *V. ventricosa.* The filament, cut and examined as a single flat sheet, shows a complex pattern in which maximum double refraction occurs when its length is arranged at 45° to the plane of vibration of the Nicols. As in the case of *V. ventricosa,* the radii of the spherical globule represent fast directions. In the whole rhizoidal process the slow direction is parallel to its length. These effects are clear cut, but at least in the latter case they result in some measure from the superimposed effect of upper and lower surfaces, for single sheets of wall (see above) reveal a more complex pattern in which there are areas with the reverse optical sign. Surface striations, like those described by Preston and Astbury for *V. ventricosa,* are easily observed. X-ray evidence on the wall of *V. ocellata* is lacking, but this organism, which produces a stout filament which can grow at the tip, offers a unique opportunity to ascertain how the wall is actually laid down. Pending a further examination of such material by X-ray methods, further speculation on the structure of this wall will be deferred. Again it will be noted that the cylindrical rhizoidal filament is not restricted in its development to any part of the vesicle, but that its emergence is determined by external conditions, e. g. light.

It is peculiar that the technique by which attached plants of *V. ventricosa* were obtained failed when applied to *V. ocellata.* Probably because it has not been successfully attached, large proliferated masses of *V. ocellata* like those which occur in nature, where they may be several inches across and often half an inch thick, have not yet been grown—although all the structures to be seen when these are dissected have been separately obtained from aplanospores. Intimate contact with a suitable substratum is such an important factor in the growth of the two species investigated that the question arises whether the attached vesicle possesses means of nutrition which the unattached ones do not have. This problem can now be investigated with *V. ventricosa,* and, until this is done, speculation concerning the possible rôle of the rhizoids or ephemeral hapteron cells as absorbing organs for the slowly expanding vesicle should be withheld. This description of the vegetative growth of *Valonia* in culture re-emphasizes the fact, already stressed by Steward and Martin (1937), that much of the physiological work done on these organisms has been based on an inadequate knowledge of their manner of growth and of their morphology.

It is generally believed that the reproduction of *Valonia* is mainly by zoospores. Neither in their habitat nor in culture has the writer ever observed zoospore formation in the species which occur at Tortugas. Aplanospore formation, however, occurs so readily and gives rise to plants so like those which grow in nature that there can be little doubt that it is an important, if not the principal, means of propagation.

It is well known that in the Valoniaceae there is a graded series of organisms which at one extreme produce within the original individual a mass of "cells" limited by individual walls (*Dictyosphaeria*), and at the other extreme large vesicles in which the protoplasm within the outer envelope is not partitioned by definite septa, e. g. *V. ventricosa*. Other species of *Valonia* are intermediate between these extremes. Most of the physiological work on *Valonia* has been based on the assumption that the organisms are simple types and the vesicles are but large single cells—a conception which, on morphological grounds, clearly presents its difficulties (see also Fritsch, 1935). Many would dismiss the difficulty by saying that the large vesicles, if not strictly homologous with single cells, behave nevertheless as self-contained physiological units. The evidence from cultural experiments is that isolated large vesicles which are only capable of limited growth may be dependent upon rhizoids and hapteron cells, and also that the protoplasm within the vesicle represents, not a single protoplast, but a relatively unstable assemblage of protoplasts which, as in the formation of aplanospores, readily fragments into its constituent units. Since *Valonia* has been so much used for physiological investigations, these considerations should be clearly recognized.

Summary

Valonia ventricosa J. Agardh and *V. ocellata* Howe have been kept alive for over two years from their original collection. Vesicles of considerable size (*V. ventricosa*) and with all the characteristics of the plant in nature have been grown attached to a suitable substratum. The development of the vesicle and rhizoids from aplanospores is illustrated by a series of photographs. *V. ventricosa* also produces filaments which penetrate the substratum and from which close clusters of vesicles arise as they do in the normal habitat. The appearance of the aplanospore and growing vesicle between crossed Nicols is described and its bearing on the structure of the wall indicated.

Valonia ocellata produces pear-shaped vesicles, cylindrical rhizoidal processes (which it is shown may become long and branched), and apparently proliferated masses composed of small cellular segments. The growth and development of all these structures from aplanospores, or the products of "segregative division," have been observed and are recorded by photographs.

Acknowledgments

The stocks of living *Valonia* were collected during 1937, when the writer enjoyed the hospitality of the Tortugas Laboratory. To the Carnegie Institution of Washington, and to the Executive Officer of the Laboratory, Dr. Tennent, the writer's thanks are due.

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A NOTE ON THE SAP COMPOSITION OF VALONIA OCELLATA HOWE AT TORTUGAS

Steward and Martin (1937) made extensive observations on the sap composition of *Valonia macrophysa* and *V. ventricosa* as they occur at the Dry Tortugas, Florida. This survey embraced a large number of collections of so-called normal sap and also samples obtained from plants after they were collected and kept in sea water unmodified as to its composition. The sap of these two species was characterized by the mean concentration of the principal components (chloride, potassium, and sodium) and the variation within the species computed from the standard deviation and the standard deviation of the mean. The range within which single sap collections would be expected to lie was set by the limits mean ± 2 × standard deviation of the population, and the range within which mean values could lie without necessarily indicating any real difference was set by the limits mean ± 2 × standard deviation of the mean. These calculations showed that there were real differences between *V. macrophysa* and *V. ventricosa* in respect of potassium and sodium content, but that there was no significant difference in their chloride content.

Collections of sap of *V. ocellata* were made in 1937. Vesicles or moruloid masses (see p. 88) were blotted with filter paper. Sap was extracted, using a large field lens, by a minute pipette, small enough to puncture single segments of the moruloid masses. Suction was not usually necessary, as the clear sap entered the capillary spontaneously. Sap samples of the order of 0.2 to 0.5 cc. were accumulated. Chloride was determined by titration of the diluted solutions with standard silver nitrate in presence of acetone, using dichloro-fluorescein as indicator. Potassium and sodium were determined spectrographically using an oxyacetylene flame by a method otherwise similar to that described by Steward and Harrison (1939).

The main result is that *V. ocellata* has a more concentrated sap than either *V. macrophysa* or *V. ventricosa*. This is shown by the figures given in table 1.

TABLE 1. *Chloride concentration of Valonia sap at Tortugas*

Species	Mean [Cl] equivs. per liter	Mean ± 2 × S. D.	Mean ± 2 × S. D. of mean
V. ocellata..................	0.704	0.790–0.618	0.732–0.676
V. macrophysa.............	0.624	0.647–0.601	0.628–0.620
V. ventricosa...............	0.628	0.650–0.606	0.631–0.625

Column 3 provides a first estimate of the probable range of composition of *V. ocellata* at Tortugas, and column 4 permits comparisons with the two other species investigated by Steward and Martin. Clearly the difference between the chloride concentration of *V. ocellata* and that of the other two species is so large that statistical computation of its reality is superfluous.

Sufficient estimates for the variation in the cation concentrations to be computed are not available. The main point is, however, that the data [K] = 0.444, [Na] = 0.400 equivs. per liter indicate that the extra chloride accumulated is not accompanied by potassium, but by sodium. Despite the discrepancy between the greatest [Cl] encountered (0.788) and the total K + Na (0.844), which is perhaps not unduly large considering the difficulties, one may conclude that the sap, like that of other *Valonias,* is mainly potassium and sodium chloride, but is richer in the latter than are the other species yet examined at Tortugas.

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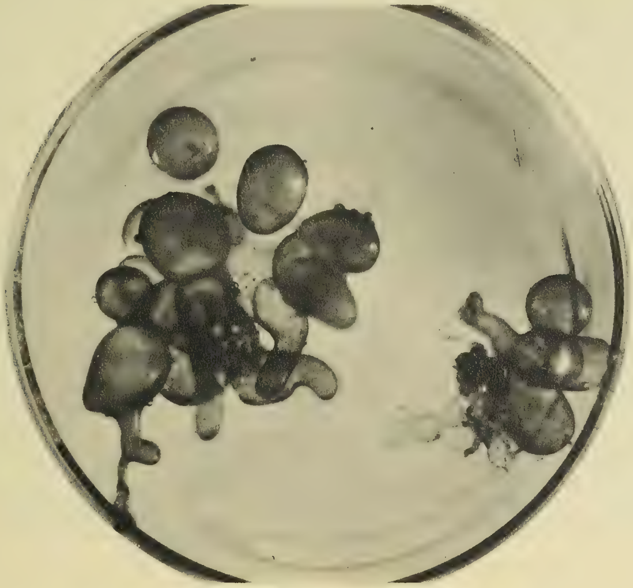


Fig. 1. Typical unsegmented vesicles of *Valonia ocellata.* × 3.

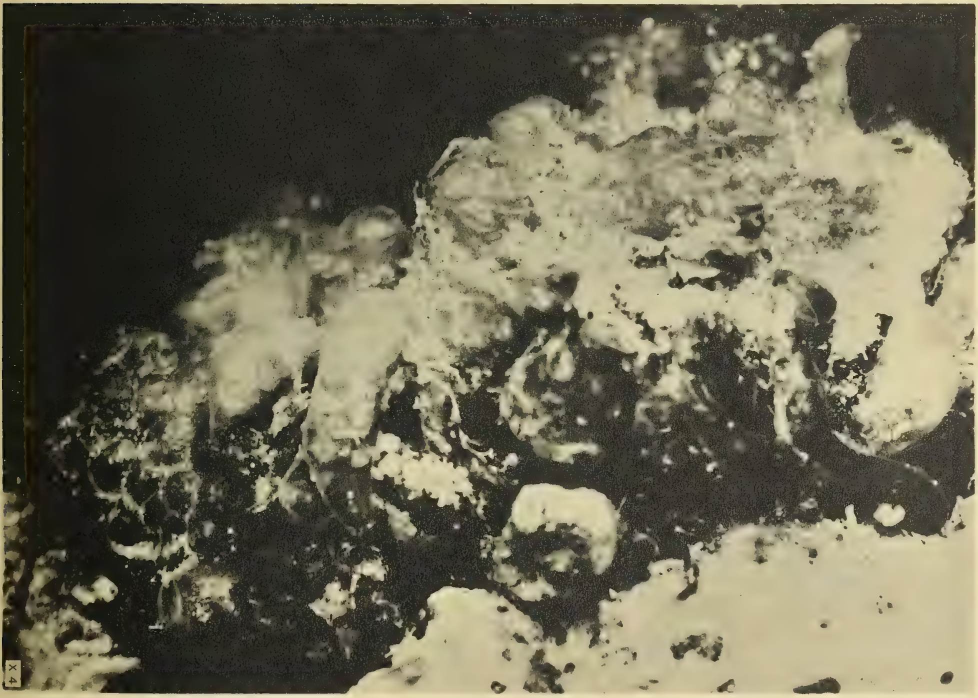


Fig. 2. *Valonia ocellata* as it grows attached to coral fragments. Specimen preserved in formalin–sea water. × 4.

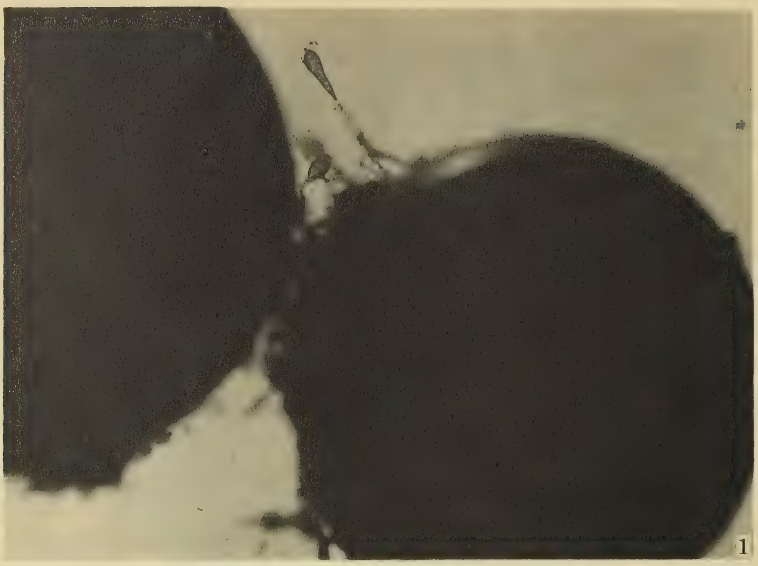


Fig. 1. Small vesicles of *Valonia ventricosa* entangled by minute rhizoidal processes, showing inflated "bulbil"-like tips. × 15.

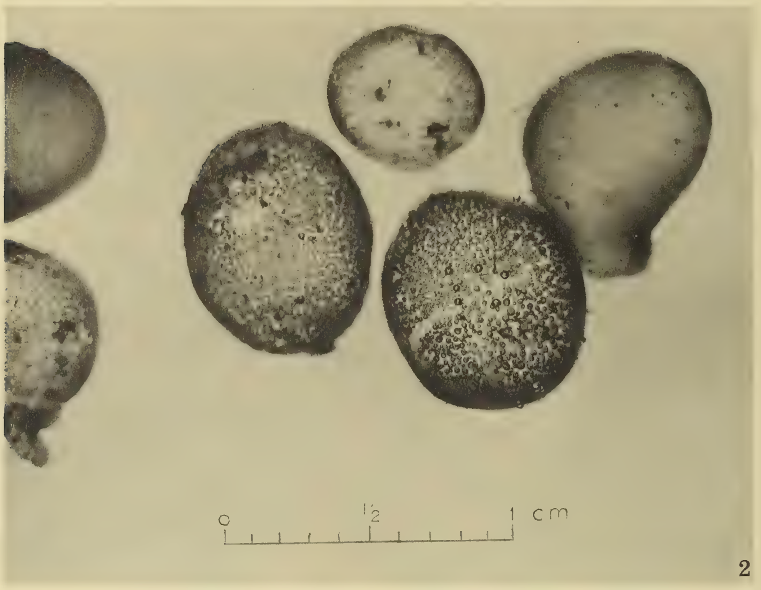


Fig. 2. *Valonia ventricosa* aplanospores inside the "parent" vesicle, and scale of centimeters.

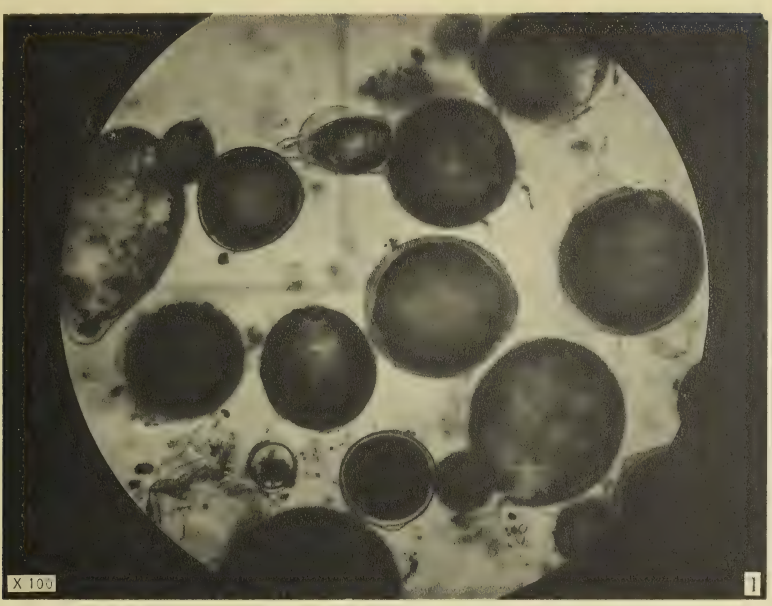


FIG. 1. *Valonia ventricosa* aplanospores, showing a rhizoid and drops of mucilage coagulated by spirit. × 100.

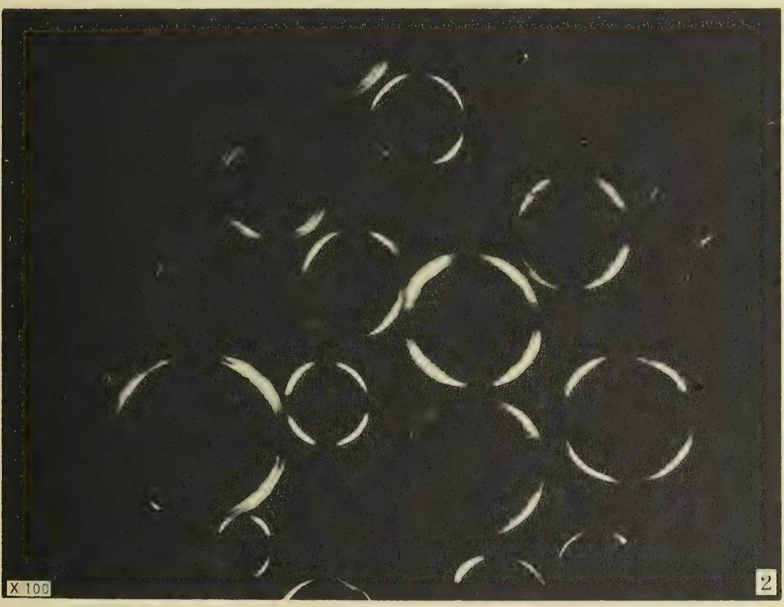
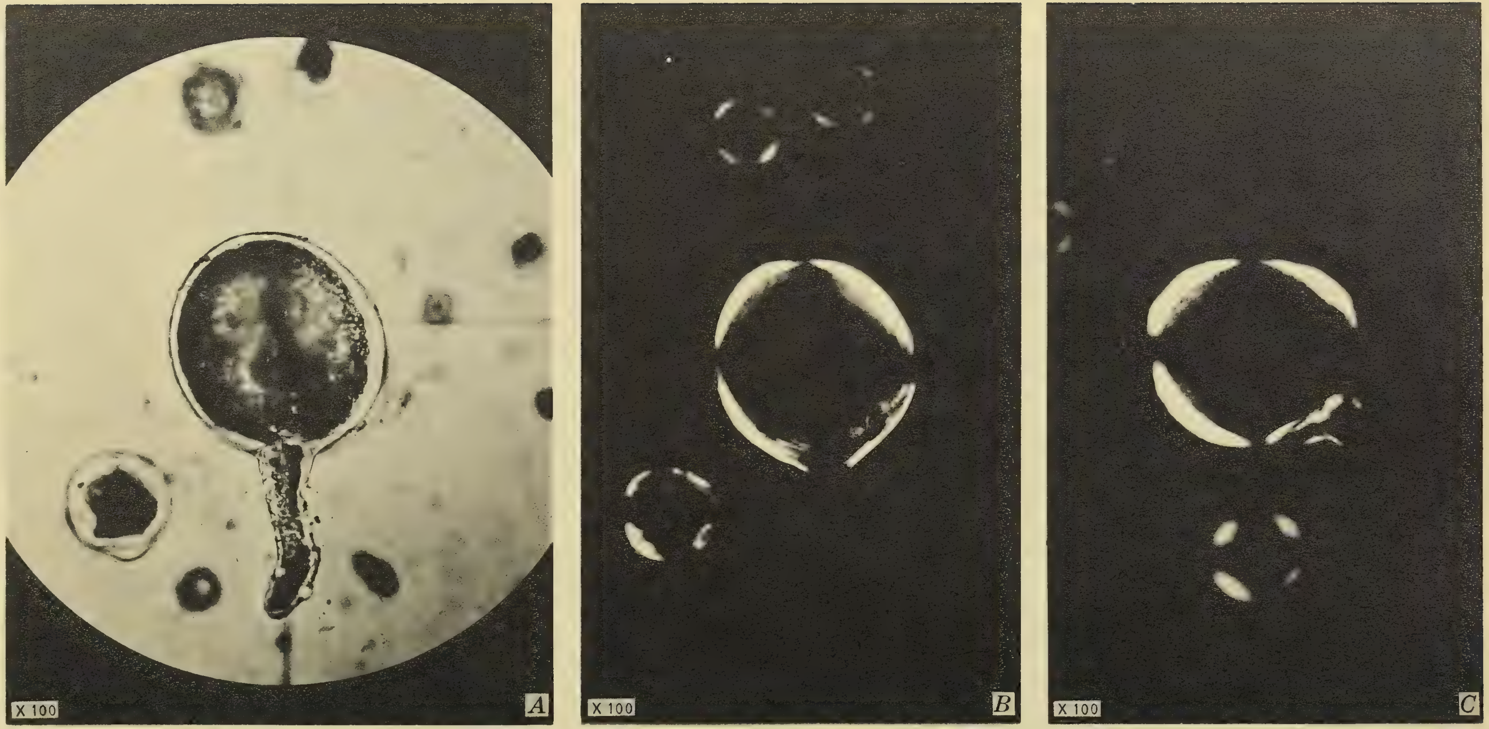
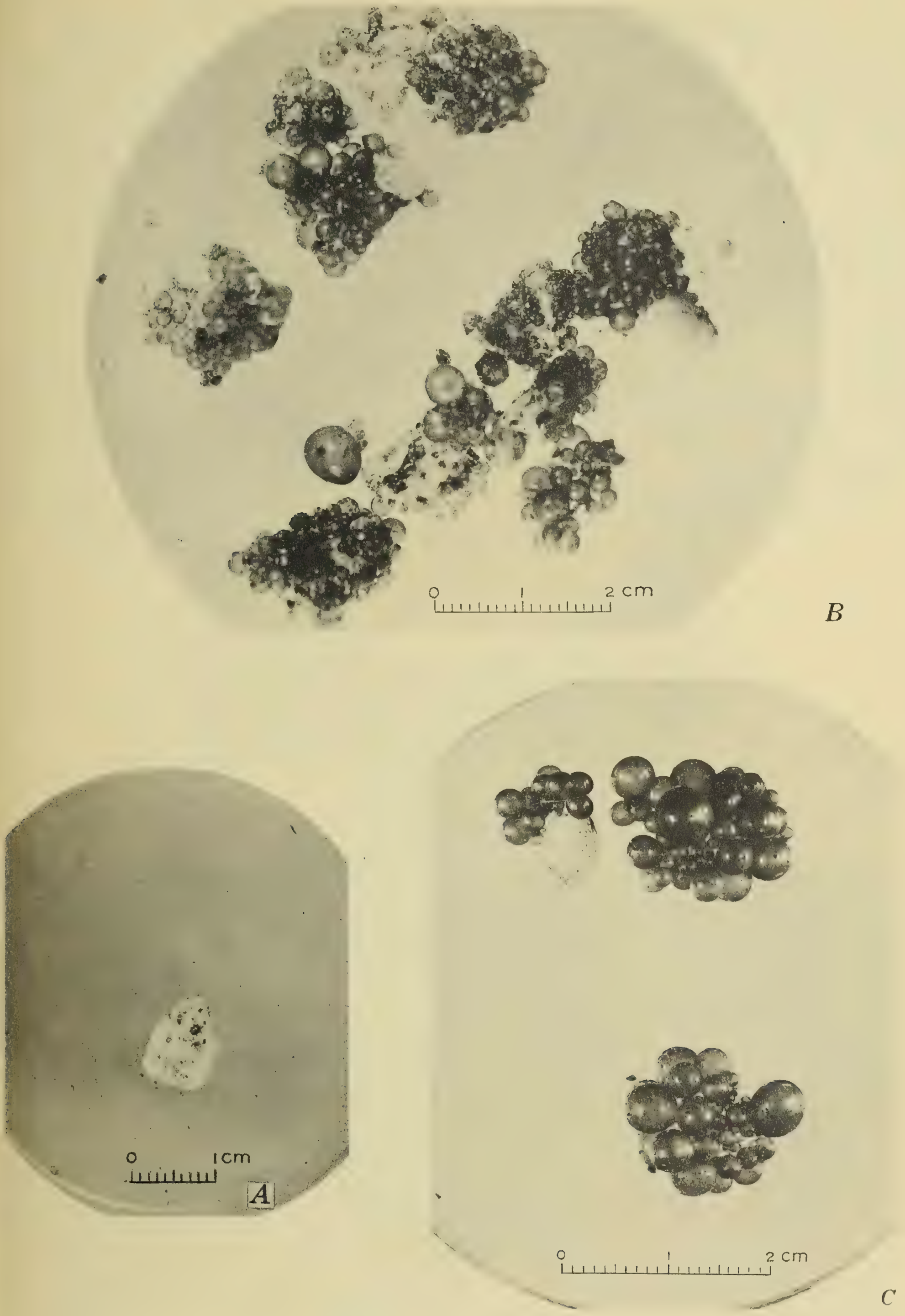


FIG. 2. *Valonia ventricosa* aplanospores between crossed Nicols. × 100.



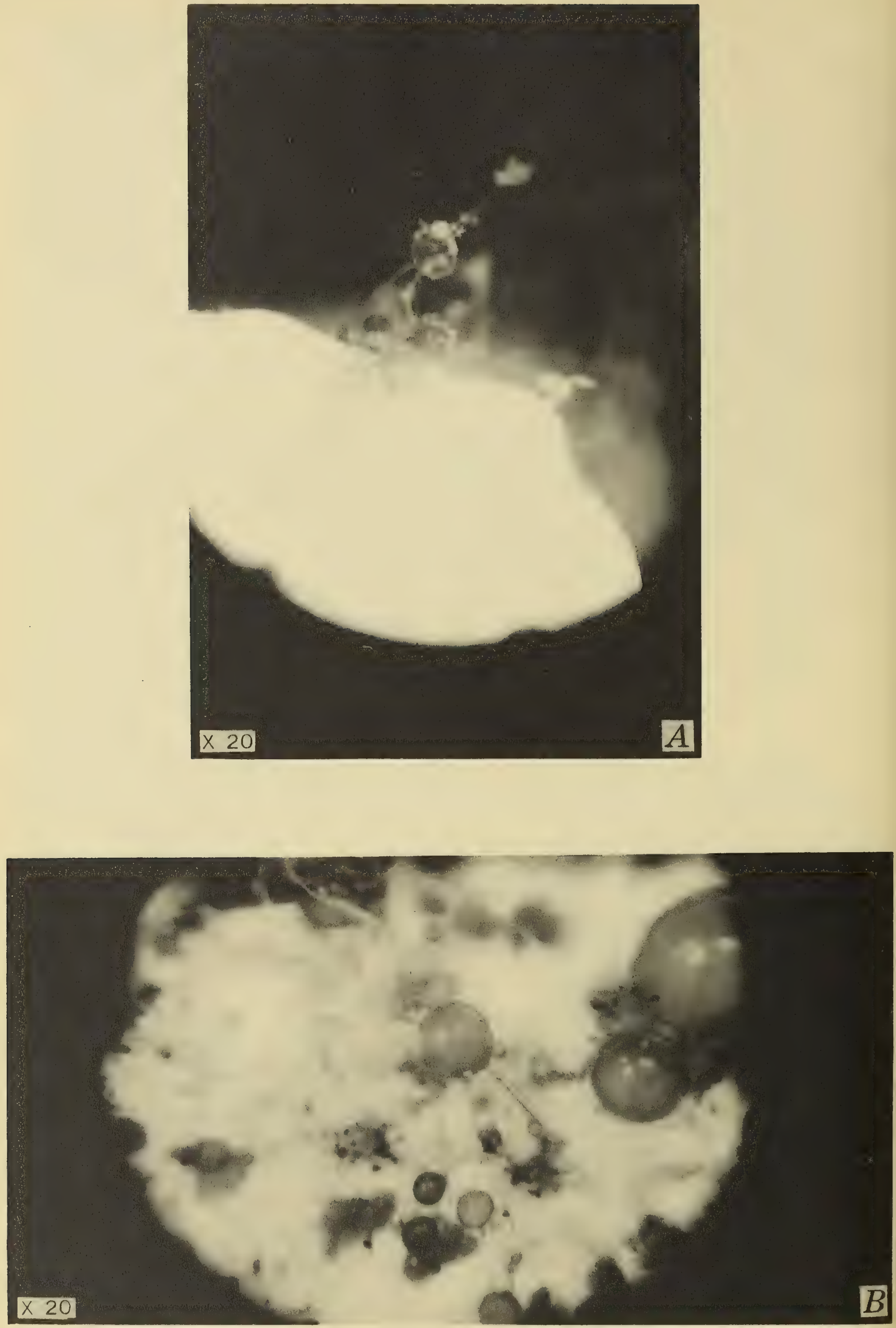
Valonia ventricosa:

A. Sporeling with rhizoid. × 100.
B. Sporeling with rhizoid between crossed Nicols—rhizoid parallel to vibration direction of one Nicol. × 100.
C. Sporeling with rhizoid between crossed Nicols—rhizoid at 45° to vibration direction of Nicols. × 100.



Valonia ventricosa growing on marble blocks:

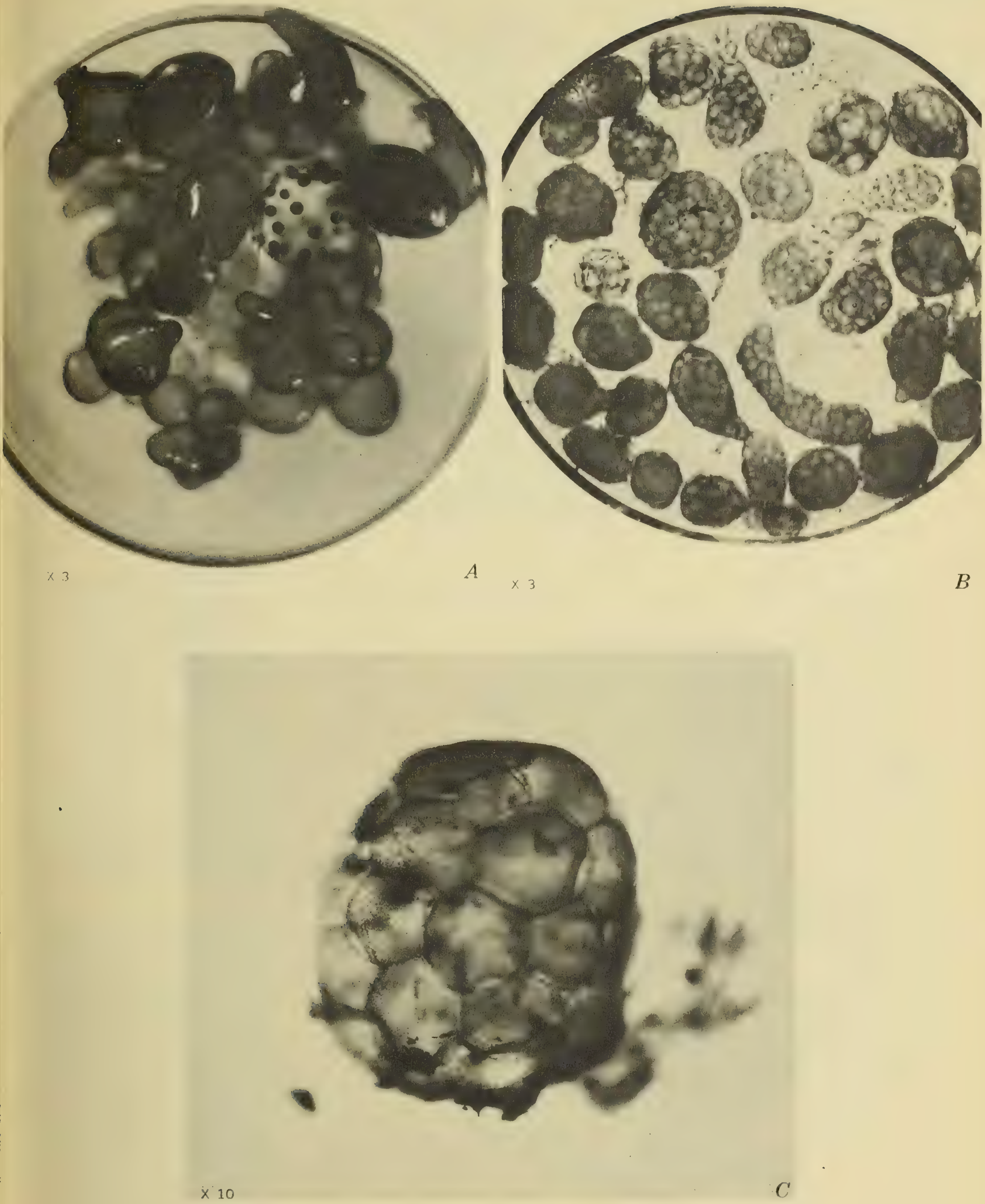
- *A.* Sporelings at about 6 weeks from "sowing," and scale of centimeters.
- *B.* Sporelings at about 6 months from "sowing," and scale of centimeters.
- *C.* Sporelings at about 10 months from "sowing," and scale of centimeters.



Valonia ventricosa growing on marble:

A. Minute vesicles with long filaments. × 20.

B. Small vesicles showing definite rhizoids. × 20.

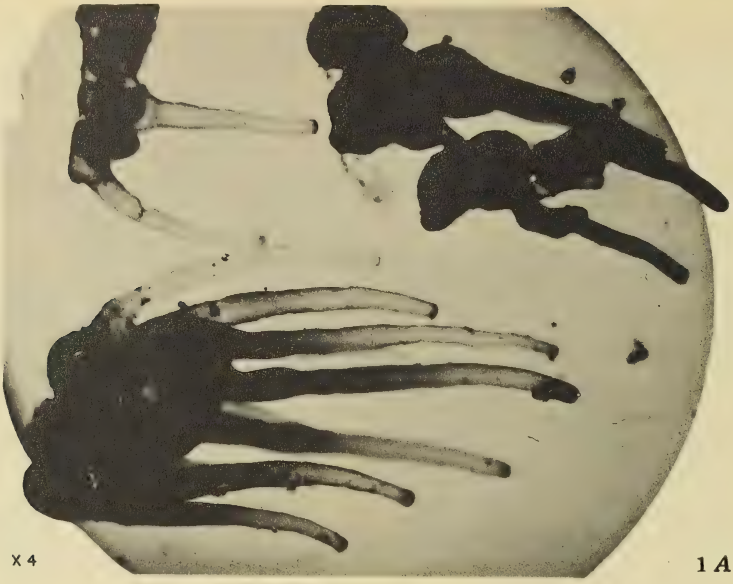


Valonia ocellata, development of segmented, "moruloid" masses:

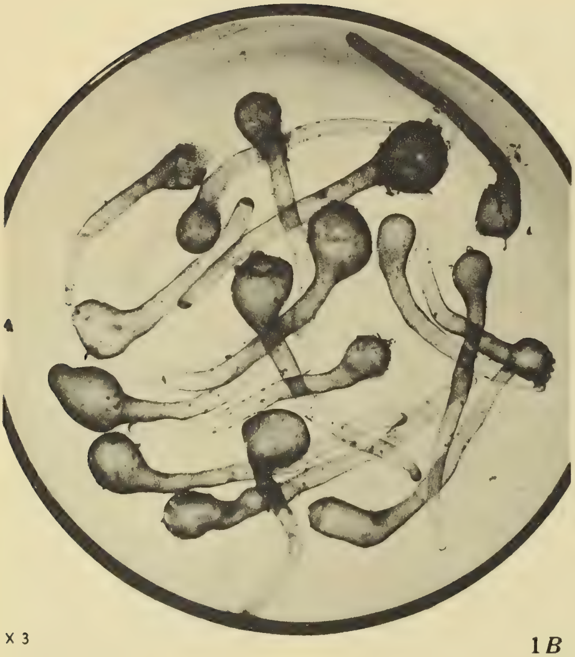
A. Large vesicles with globular sporelings formed 2 to 3 months from collection. × 3.

B. Typical "moruloid" masses grown from aplanospores within the old vesicle. × 3.

C. Structure developed by mutual compression of globular spores within the old vesicle, the wall of which is still attached (right). × 10.



1 *A*



1 *B*

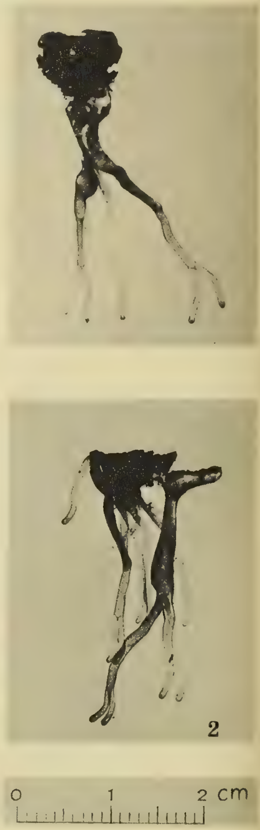

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Fig. 1. *Valonia ocellata,* sporelings with cylindrical, filamentous rhizoidal processes:

A. As developed by unilateral, artificial illumination. × 4.

B. Individuals dissected to show spherical and cylindrical portions without septa. × 3.

Fig. 2. *Valonia ocellata* with dichotomously branched rhizoidal processes, and scale of centimeters.

VI

The Effect of Light on the Inherent E.M.F. of Valonia ventricosa

II. The Relative Energy Absorption Spectrum

By Gordon Marsh

Zoological Laboratories, State University of Iowa

With three text figures

[ISSUED MAY 16, 1940]

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The Effect of Light on the Inherent E.M.F. of Valonia ventricosa

II. The Relative Energy Absorption Spectrum[1]

INTRODUCTION

The interest in the relative effectiveness of different wave-length bands of visible light upon bioelectric potentials centers about the question of the nature of the agent in the living cell which absorbs the radiant energy, and its contribution to the electromotive mechanism. For the green plants three principal lines of evidence have been adduced to support the conclusion that chlorophyll is the photosensitive agent: (1) the comparison of the effect of light on the potential of chlorophyll-bearing and chlorophyll-free plant structures; (2) the effect on the potential of light of varying spectral distribution; and (3) the effect of carbon dioxide. This latter aspect of the problem will be discussed in the third paper of this series.

Haake (1892) was the first to show that electrical changes produced in green leaves by illumination were absent in etiolated leaves and in chlorophyll-free structures. He concluded that chlorophyll was essential to the electromotive action of light. This was confirmed by A. D. Waller (Stern, 1924, p. 170; the original work was not available to the writer). J. C. Waller (1925, 1929) arrived at the same conclusion from experiments with CO_2 and from the fact that light produced no electrical change in etiolated bean and mustard seedlings. He likewise found albino leaves, albino areas in variegated leaves, and chlorophyll-free petals to be unresponsive. Glass (1933) reported illumination of the onion root tip to be without effect upon the inherent potential.

On the other hand, Bose (1907) obtained an electrical response to light from etiolated celery stem (confirmed by J. C. Waller, 1929). Bose also reported strong responses from petals of *Sesbania* and *Eucharis,* and stated that in his experiments there was no correlation between the magnitude of the response and the chlorophyll content. He concluded that the electrical response to light was fundamentally the same as that induced by other stimuli. Sheard (1929, 1930) found visible light to be virtually without effect upon leaves of sunflower and poinsettia. More recently Clark (1935) obtained a pronounced, if somewhat peculiar, response from the etiolated oat coleoptile, the electrical change occurring in the dark at the end of a ten-minute period of illumination.

It is evident that the conclusions of neither Haake nor Bose have general application. There is, however, no real conflict between the experimental results. It was emphasized in the first paper of this series (Marsh, 1939) that a distinction must be made between the transient (transitional) effects of illumination and the steady state effects. Only from the latter can a consistent relation be demonstrated between E.M.F. and light intensity. The results of experiments on chlorophyll-free structures have all been transient effects and are rightly placed in the category of stimulus-response phenomena. The results with chlorophyll-bearing structures have in many, but not all, instances been steady state relations. Choice of material and experimental technique determine which type of change will appear. Whether a part of the steady E.M.F. produced by illumination in green plants is due to absorption of light by an agent other than chlorophyll cannot be settled on the basis of this mode of investigation.

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[1] Aided by a grant from the Rockefeller Foundation for work in cellular physiology.

Relatively few attempts have been made to control the spectral quality of the incident light. A. D. Waller (1900) compared the action of light transmitted by glass filters and found the magnitude of the electrical change to decrease in the order: white, red, blue, green. Similar experiments by J. C. Waller (1925) yielded the order: red, orange, yellow to violet, the latter range having but a small effect. Earlier workers had found this same order of effectiveness of colored lights on photosynthetic rate. Neither of these investigators stated the transmission characteristics of the filters used, nor attempted to control or correct for relative energies. Sheard (1929, 1930) found only the ultraviolet and infrared to be effective in altering E.M.F. Brown (1938) measured the magnitude of the electrical change in *Chara* internodes exposed to light of different spectral quality and variable total energy transmitted by Corning glass filters. The decreasing order of effectiveness was: white, red (649–740 mμ), light blue (445–505 mμ), dark blue (415–490 mμ), green (490–555 mμ).

These effects of light of different wave-length regions on the E.M.F., especially those reported by Brown, are qualitatively in the direction to be expected if the energy is absorbed by the chlorophyll system. (Sheard's results constitute an exception; a criticism of his work has been offered [Marsh, 1939].) Quantitatively, the magnitude of the E.M.F. was determined by factors not readily separable: the absorption spectrum of the photosensitive material; the total energy incident upon the living system; and the form of the relation of the E.M.F. to light intensity. A critical proof of the intervention of chlorophyll would appear desirable.

Such a proof is believed to be presented in the present paper. The effect of different wave-length bands of the visible spectrum upon the protoplasmic E.M.F. of impaled *Valonia ventricosa* was determined by a method which separates the factors mentioned above. A relative energy absorption spectrum is obtained which compares favorably with the corresponding spectrum for chlorophyll mixtures calculated from the data of Zscheile (1934).

Method

The preparation of the material, the details of the measuring circuit, and the general conditions of illumination have been described (Marsh, 1939). The

Valonia were collected from a shallow pool on the protected side of Bush Key Reef, where the substratum was a loose coral "rubble" supporting a considerable population of other algae. In the results below there is evidence that members of a population of cells possess variable absorption capacities for light. The variation may be due to differences in concentration of chlorophyll correlated with conditions of exposure to light during growth of the cells, as has been found for other plants. Collections of *Valonia* from this location included as extremes cells which had grown exposed to full sunlight and others which had been well screened beneath other vegetation and the coral fragments.

The light source was a 500-watt Mazda floodlight with a rated output of 8650 lumens at 115 volts. The *Valonia* were contained in a light-proof box equipped with a shutter for insertion of the filters. Two plane mirrors at the rear of the box provided nearly uniform illumination from three directions. Light intensities were calculated in foot-candles from the rated output of the lamp and the sum of the three light paths, using the inverse-square law. A record was kept of the voltage across the lamp and the intensity was corrected for small voltage changes on the assumption that the intensity was proportional to the square of the voltage. This is approximately true for the wattage between 105 and 125 volts (Walsh, 1926, pp. 481–483). In general the voltage was held near 115 volts and the above limits were rarely reached.

The filters were Wratten gelatin films, 3 × 4 inches, mounted between glass plates. They are listed, with their transmission ranges, in table 1. In the calculations involving the filters the percentage transmissions for different wave lengths were taken from the manufacturer's catalogue (*Wratten light filters,* Eastman Kodak Co., 14th ed., 1936).

Two procedures may be followed in testing the relative effectiveness of different spectral regions: (1) the magnitude of the E.M.F. may be compared for radiations of equal energy content; or (2) the energy content of radiations producing the same E.M.F. may be determined. The first method is subject to the criticism offered in the introduction; the second, which eliminates this difficulty, is the one used in the present paper. From the photochemical standpoint the same magnitude of effect should be produced when the energy absorption per unit time from lights of different spectral quality is the same. Provided the steady E.M.F. produced in a *Valonia* by illumination at a given intensity of colored light is matched for white light, the absolute magnitude of the E.M.F. matched need not be the same for each filter. This is convenient for the present problem, as the time element involved in producing the same E.M.F. in the same cell with all the filters would be prohibitive, and a given E.M.F. so produced in different cells would not necessarily represent an identical effect. From the intensities producing an E.M.F. match, combined with the known spectral distribution of radiant energy from the source and transmitted by the filters, a relative energy absorption spectrum of the photochemical system can be calculated which will be quantitative within the limits of accuracy of determination of intensities and energy distributions. The method is a somewhat elaborate version of that employed by Hecht (1921, 1928).

The analysis is limited to the region of the visible spectrum between 400 and 700 mμ, principally because transmission data are available for the filters only between these limits. The filters all transmit freely in the infrared. The radiation passed through about 3 centimeters of sea water before reaching the *Valonia,* so that wave lengths longer than 1200 mμ were absent (Brackett, 1936, p. 126). The source had a small emission between 300 and 400 mμ, which was considerably reduced by the glass wall of the lamp itself and the pyrex containing vessel. Filter no. 2 (aesculine) transmits to 380 mμ. The possible effect of ignoring these spectral regions will be discussed later.

Intensity Ratios for Filtered Light

That the effect of absorbed light of all wave lengths on the E.M.F. is the same, the magnitude of effect being determined only by the amount of energy absorbed, is implied by the results for filter no. 12 (minus blue) shown in table 1. The last four values were obtained from a single *Valonia.* After the E.M.F. had become constant at 9 millivolts in the dark, the cell was exposed to light from the source at 35.7 foot-candles intensity. The potential became steady at 14.3 millivolts. Filter no. 12 was then interposed and the intensity increased (to 102 foot-candles) until the steady level of E.M.F. was nearly the same as with the unfiltered source (14.5 mv.). The filter was removed and the matching process repeated at three higher intensities. The ratio of intensities of unfiltered to filtered light giving a match in E.M.F. is nearly constant (table 1, column 7) and is independent of the absolute intensity, as well as of the absolute value of the E.M.F. matched. If the increase in steady E.M.F. above the dark potential is plotted against the logarithm of the light intensity for this experiment, two curves are obtained, separated on the abscissa by a nearly constant interval. Both are entirely similar in form to the sigmoid ascending limbs of the full E.M.F. : log intensity curves previously described (Marsh, 1939), so that a change in spectral quality of the illumination can be compensated for by a definite change in quantity (intensity). No attempt was made to obtain similar data covering any extensive range of intensities for the other filters. The same inferences may be drawn, however, from the results shown in table 1 below for filters 2, 31, 12-31, 43, and 89.

Figure 1 gives a portion of the time record of the E.M.F. of three cells during the process (which, as a whole, is trial and error) of obtaining an E.M.F. match with filtered and unfiltered light. The first 15 minutes of curve 1 (the scale on the abscissa should be multiplied by 2 for this curve) shows the steady level of E.M.F. for light passing filter no. 43 (minus red 2, 400–610 mμ) with the source at 270 foot-candles intensity. The intensity was then increased to 675 foot-candles. A characteristic transition curve of E.M.F. followed, the potential difference increasing to 23.5 millivolts in some 80 minutes. A brief period of constancy was taken to indicate that steady level had been reached; the filter was removed and the intensity reduced to 108 foot-candles. The subsequent rise and fall was due principally to the fact that removal of the filter exposed the cell for a brief period to unfiltered light at 675 foot-candles. In about an hour and a half the potential became steady, the terminal value being 23.4 millivolts. This curve illustrates one extreme of accuracy of determination of the matching intensity, for it is probable that the E.M.F. had not reached a steady level for the filtered light. This source of error was minimized in the majority of the determinations by avoiding large intensity changes, thus reducing the time necessary to reach a steady level (Marsh, 1939).

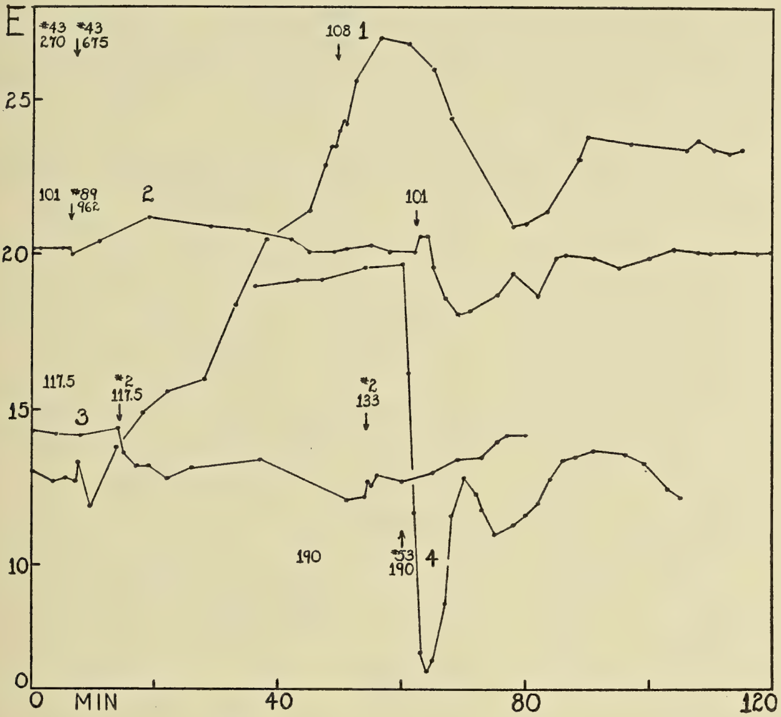

Fig. 1. A portion of the detail of the trial-and-error process of obtaining an E.M.F. match with filtered and unfiltered light. Curve 4 shows the transition curve of E.M.F. produced by interposing the transmission filter no. 53 in the light path.

Curve 2 of figure 1 records a more accurate match for filter no. 89 (signalling red [light], 650–700 mμ) at 962 foot-candles. The steady E.M.F. with white light at 101 foot-candles before and after exposure with the filter was within 0.1 millivolt of the steady level with the filter. Curve 3 shows the matching process for filter no. 2 (aesculine, 400–700 mμ). The E.M.F. was steady at 14.4 millivolts with the light at 117.5 foot-candles intensity. Interposition of the filter produced a typical intensity-reduction curve with the loss of about 2 millivolts potential. The light intensity was then increased to 133 foot-candles; this restored the E.M.F. to 14.2 millivolts.

The time curves of transition of E.M.F. between steady levels shown in figure 1 are entirely similar in form to those reported in the first paper of this series (Marsh, 1939) and are evidently determined by the same factors. Curve 4 illustrates the same principle. Interposition of filter no. 53 (naphthol green 3, 480–620 mμ) in the light path at 190 foot-candles intensity produced a time change in E.M.F. practically equivalent to reducing the intensity to zero. This filter has a low total transmission and, as will be seen below, the electromotive system has a low absorption for the wave band transmitted. The characteristics of the transition curves of E.M.F. between steady levels are not dependent upon the spectral quality of the incident light. This is additional evidence that the changes are determined by the total energy absorbed.

A summary of the data obtained with eight filters and two filter combinations is contained in table 1. The limits of the transmission ranges are stated in millimicrons in column 2. Columns 3 and 5 give the steady E.M.F. matched in millivolts at the intensities in foot-candles shown in columns 4 and 6 for light from the source and light passing the filter respectively. The ratio of the in-

TABLE 1. *Ratio of intensity of unfiltered to that of filtered light producing the same steady E.M.F.*

1	2	3	4	5	6	7
Filter no.	Range (mμ)	Unfiltered		Filtered		Intensity ratio
		Mv.	Ft.-candles	Mv.	Ft.-candles	
2, aesculine	400–700	18.0 14.4	169.0 117.5	18.0 14.2	192 133	0.88 .885
3, aero 1	450–700	18.8	128.0	18.8	221	.58
12, minus blue	500–700	14.2	96.0	14.2	166	.578
		14.3 15.4 22.3 25.8	35.7 88.1 159.5 244.0	14.5 15.8 22.3 25.9	102 232 396 617	.35 .38 .403 .396
31, minus green	400–490 590–700	23.1 21.2 23.3	204.0 158.0 208.0	23.5 20.8 23.7	295 195 247	.706 .81 .842
12-31	590–700	11.3 13.7	121.0 108.0	11.1 13.9	247 290	.49 .372
43, minus red 2	400–610	23.4 8.8 16.7	108.0 34.8 98.5	23.5 9.0 16.2	675 431 547	.16 .081 .18
47-C5, projection blue	400–530	18.1	98.5	18.7	720	.137
31-47	400–490	14.8	76.2	14.8	2095	.0364
53, naphthol green 3	480–620	17.1	22.0	17.3	1050	.0209
89, signalling red (light)	650–700	20.2 17.5	101.0 77.0	20.1 17.0	962 552	.105 0.139

tensity of the unfiltered to that of the filtered light for each E.M.F. match appears in column 7. With the exception of filter no. 12, each ratio represents an individual experiment.

In the majority of instances the E.M.F. was matched for filtered and unfiltered light to within 0.2 millivolt. The extreme deviation is 0.6 millivolt for filter no. 47. A number of other determinations of the intensity ratio were discarded because later examination of the record of an apparent match disclosed that the E.M.F.'s with filtered and unfiltered light deviated by a millivolt or more. The character of the deviations permits the determinations to be listed for purposes of comparison: no. 2: > 0.74; no. 12: > 0.36; no. 43: > 0.123, > 0.077; no. 47: < 0.136, < 0.134; no. 89: < 0.154, < 0.135. Two experiments with filter no. 53 and one with no. 43 were omitted because the E.M.F. underwent continuous fluctuation with the filtered light and did not approach a steady state.

In general, the smaller the value of the ratio, the greater was the variation in a set of values for a given filter. This is to be expected from the form of the E.M.F. : log *I* curve for *Valonia* (Marsh, 1939, fig. 2). Below about 100 foot-candles a large change in light intensity may produce a shift in the steady level of E.M.F. of only a fraction of a millivolt. Between 100 and 200 foot-candles a comparable change in intensity produces a relatively large change in steady E.M.F. Values of the intensity ratio obtained with the intensity of the unfiltered light of the order of 100 foot-candles or more should therefore be more accurate than those obtained with the intensity of unfiltered light less than 100 foot-candles. For the filters for which the intensity ratio was small, the matching intensity with unfiltered light tended to be less than 100 foot-candles. This point was not properly appreciated at the time the experiments were performed; it is probably the largest single source of error in the determination of the relative effectiveness of different spectral regions transmitted by the filters.

The deviation of the single value of 0.578 for filter no. 12 from the average of the values obtained in the second experiment is rather larger than would be expected from the high transmission of this filter. The close grouping of the four values from the one experiment suggests either some systematic error of procedure or that the absorption characteristics of individual *Valonia* may show moderate differences. The latter alternative would appear the more probable.

The Relative Energy Absorption Spectrum of the Electromotive System

Provided that the absorption of light is a quantum process, as is true for photosynthesis (Warburg and Negelein, 1923), an E.M.F. match will be obtained for a given *Valonia* cell illuminated with light from the source and with light passing a filter when the number of light quanta absorbed per unit time is equal under the two conditions. The number of quanta absorbed will be the product of the incident intensity (expressed in quanta) and the fraction of the incident energy absorbed. For light composed of a mixture of different wave lengths the latter quantity may be expressed as the average value of the energy fraction absorbed at each wave length, A. If we let the subscripts u and f refer respectively to the unfiltered source and to the filtered light, then at any E.M.F. match

$$A' = \frac{A_f}{A_u} = \frac{Q_u}{Q_f}\frac{I_u}{I_f} \,. \tag{1}$$

A' is the average energy fraction absorbed over a given spectral region (transmitted by a filter) relative to the similar average over the entire spectral region considered (400–700 mμ); it will be referred to as the relative energy absorption. Q is the intensity in quanta/sec./cm.^{2}/steradian emitted by the source (or as modified by the filters), and is determined graphically from the distribution plots below. I is a factor correcting the intensity for distance of the cell from the source. The values of the ratio I_u/I_f are numerically the same as the intensity ratios in table 1.

The energy distribution[2] of the source with wave length as calculated from Wien's law upon the assumption of a black-body temperature of 2900° K for the 500-watt lamp (Forsythe and Watson, 1932) is shown as curve 1 of figure 2. The other curves, designated by the filter numbers appearing in table 2, give the energy distribution of the light from the source transmitted by the filters. The average ordinate for each 5-mμ interval was multiplied by the average number of quanta per erg for that interval and the interval areas summed for each curve. The quantity Q_u/Q_f, which may be termed the quantum area ratio, was then obtained by dividing the area for the source by the area for each filter or filter combination.

The ratios appear in column 3 of table 2. The fourth column shows the intensity ratios for the filters, as the arithmetic means of the individual ratios listed in table 1, column 7. For filter no. 12 two values are given: that in parentheses is the mean of the five determinations in table 1; for the first value the mean of the four determinations on one cell is averaged with the value from the other cell. The latter procedure would appear to be the correct one, but both values are used in what follows. The product of the quantities in columns 3 and 4 is the relative energy absorption over the transmission range of the filter and appears in column 5.

It follows from Beer's[3] law that the fraction of the incident energy of a given wave length absorbed by a substance in homogeneous solution will be nearly proportional to the absorption coefficient, provided the product of the concentration of the substance and the length of the light path is sufficiently small. The thickness of the protoplasmic layer in *Valonia* is about 8×10^{-4} cm.

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[2] While the intensity was corrected for voltage changes, no attempt was made to perform the more laborious companion corrections for changes in energy distribution.

[3] There is no implication that Beer's law holds for absorption of light by chlorophyll in the living chloroplast, a condition which may be doubted. Provided that no great distortion of the absorption spectrum occurs because of conditions in the chloroplast, and provided that no large variation in concentration of chlorophyll exists among members of a population of *Valonia*, whether Beer's law holds is a matter of indifference. The relation and the accompanying figures are cited to show that the comparison to follow has a reasonable basis.

(Doyle, 1935); the "proportionality constant" will vary by less than 25 per cent of its maximum value if the absorbing substance (chlorophyll) does not exceed 0.375 per cent of the wet weight. For a given spectral region the relative energy absorption, *A′*, will similarly be nearly proportional to an analogous quantity, the relative average absorption coefficient over the same region (termed, for convenience, the relative absorption). The relative absorption was calculated from the absorption spectra of chlorophylls *a* and *b* in ether determined by Zscheile (1934, p. 541). The areas under the two curves were obtained over the

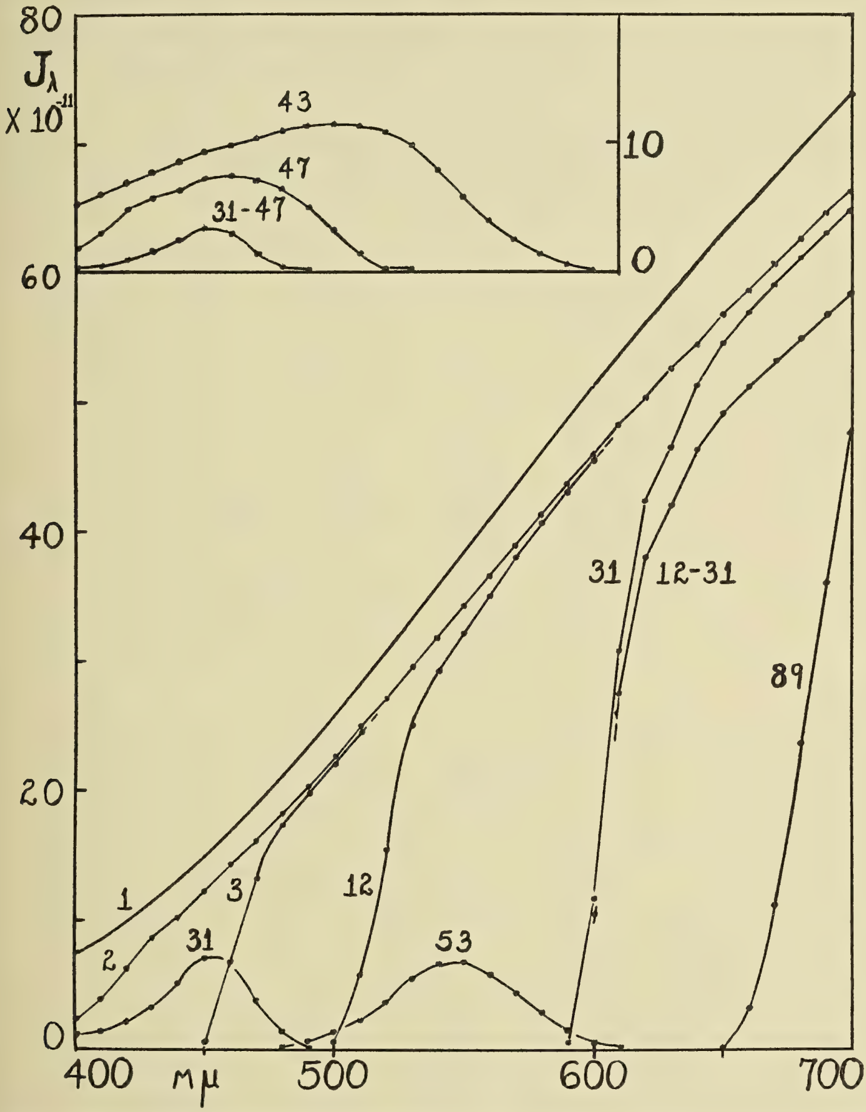

FIG. 2. The energy distribution of the source (curve 1) and of the source as transmitted by the designated filters. The ordinate is the radiance, the abscissa the wave length in millimicrons.

spectral ranges of the filters and over the entire region 400–700 mμ. Three times the *a* values were added to the *b* values and the sums for the filter ranges divided by the sum for the entire range to give the relative absorption of a mixture of the two chlorophyll components in the ratio $a/b = 3$, which appears to be characteristic of many plants (Willstatter and Stoll, 1913; Winterstein and Stein, 1933). The values are listed in column 6 of table 2. Column 7 shows the relative absorption of a mixture of equal parts of chlorophylls *a* and *b*.

Table 2. *Comparison of the relative energy absorption by the electromotive system for light passing the filters with the relative average absorption coefficient of chlorophyll mixtures over the same spectral regions*

1 Filter no.	2 Range (mμ)	3 Q_u/Q_f	4 I_u/I_f	5 A'	6 Relative absorption by chlorophyll	7
					$a/b = 3$	$a/b = 1$
31-47	400–490	103.3	0.0364	3.76	1.94	2.08
31	400–490 590–700	1.99	.785	1.56	1.393	1.393
47	400–530	25.55	.137	3.5	1.39	1.48
89	650–700	10.47	.122	1.275	1.25	0.975
2	400–700	1.115	.8825	0.985	1.0	1.0
43	400–610	9.25	.14	1.295	0.974	1.055
12-31	590–700	2.3	.431	0.991	0.945	0.835
12	500–700	1.31	.48 (.422)	0.629 (0.553)	0.62	0.56
3	450–700	1.165	.58	0.676	0.604	0.65
53	480–610	29.5	0.0209	0.618	0.265	0.259

The filters are arranged in table 2 in the order of decreasing magnitude of the relative absorption for the chlorophyll mixture $a/b = 3$. This order is only roughly maintained by the A' values in column 5. Rearrangement of the filters to correspond to a decreasing order for the relative absorption of the mixture $a/b = 1$ (column 7) results in a much better agreement, filter no. 89 being the only one out of order. The A' values in table 2 fall into two groups with respect to their correspondence to the relative absorption values; with the exception of filter no. 89, an identical grouping may be made on the basis of the transmissions of the filters. (The quantum area ratios in column 3 are an approximate inverse measure of the transmissions of the filters.) Filters nos. 31-47, 47, 43, and 53 have low transmissions; the A' values are 33 to 152 per cent greater than the corresponding relative absorptions in column 6. Filter no. 89, with a low transmission, shows surprisingly good agreement. Filters nos. 31, 2, 12-31, 12, and 3 have high transmissions; the A' values range from 1.5 per cent lower to 12 per cent greater than the corresponding values in column 6. It seems probable that the discrepancies are principally accounted for by errors in the determination of the intensity ratios for the filters, which are greater for the filters of low transmission. With the exception of filter no. 2 the A' values are all too high. This might imply some systematic error of procedure were not the deviations for filters nos. 89 and 12 (first value) so small as to indicate that the error lies in the experimentation rather than in the analysis. The close correspondence of the A' value for filter no. 2, which has the highest transmission and for which the inherent errors should be least, is an assurance that the general method employed is a valid one.

The four main absorption peaks for chlorophylls *a* and *b* were found by Zscheile (1934) to lie at the wave lengths 427.5, 452.5, 642.5, and 660 mμ. The absorption minimum for both components together lies between 480 and 600 mμ.

TABLE 3. *The relative energy absorption by the electromotive system compared with the relative absorption of chlorophyll mixtures over the five adjoining regions of the visible spectrum which contain the four absorption maxima and the absorption minimum of the chlorophyll spectrum*

1	2	3	4	5
Spectral region	Filter (T=transmission) (E=exclusion)	A'	Relative absorption by chlorophyll	
			$a/b = 3$	$a/b = 1$
400–450	3E	*2.62*	2.97	2.76
400–500	12E	1.742 (1.894)		
400–590	12-31E	1.005		
450–500	3E, 12E	*0.864* (1.17)	0.537	1.007
450–700	3T	0.676		
500–590	12E, 12-31E	*0.187* (0.018)	0.1987	0.223
500–700	12T	0.629 (0.553)		
590–650	12-31T, 89T	*0.754*	0.683	0.721
590–700	12-31T	0.991		
650–700	89T	*1.275*	1.25	0.972

The transmission ranges[4] of the short-wave cut-off filters nos. 3, 12, 12-31, and 89 make it possible to divide the spectrum between 400 and 700 mμ into five regions: 400–450, 450–500, 500–590, 590–650, and 650–700 mμ. These spectral regions approximately isolate the separate absorption peaks and the absorption minimum, and thus offer a convenient basis for the construction of a relative energy absorption spectrum which may be compared with the corresponding values of the relative absorption by chlorophyll.

Since $A'_{400-700} = 1$, there can be obtained from each filter two A' values: one, the calculated value for the spectral region transmitted; the other, a calculated value for the region excluded. For an example we may use the data for filters nos. 3 and 12. The transmission band for no. 3 (450–700 mμ) is 250 mμ in extent and the $A'_{450-700} = 0.676$. The product 0.676 × 250 added to $50 \times A'_{400-450}$ is equal to $A'_{400-700} \times 300$, or $300 - 169 = 131 = 50A'_{400-540}$, from which $A'_{400-450} = 2.62$. In a similar manner with filter no. 12 we obtain $100A'_{400-500} = 300 - (200 \times 0.629) = 174.2$ and $A'_{400-500}$ 1.742. The combination of these values gives $50A'_{450-500} = 174.2 - 131 = 43.2$ and $A'_{450-500} = 0.864$.

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[4] It may not be superfluous to point out that the transmission characteristics, which make this the only useful filter set for the purpose, are the sole basis for their selection.

The A' values obtained for the regions transmitted and excluded by the four filters together with the combinations of these values which yield the relative energy absorption over the five spectral regions listed above appear in the third column of table 3. The first column shows the spectral region. The second column shows the filter by means of which the region is isolated; the letters following the filter number indicate whether the region is transmitted or excluded. Where two filters appear in this column, the A' value obtained corresponds to that for $A'_{450-500}$ in the above example. For filter no. 12 the values in parentheses were secured through the use of the A' value given in parentheses

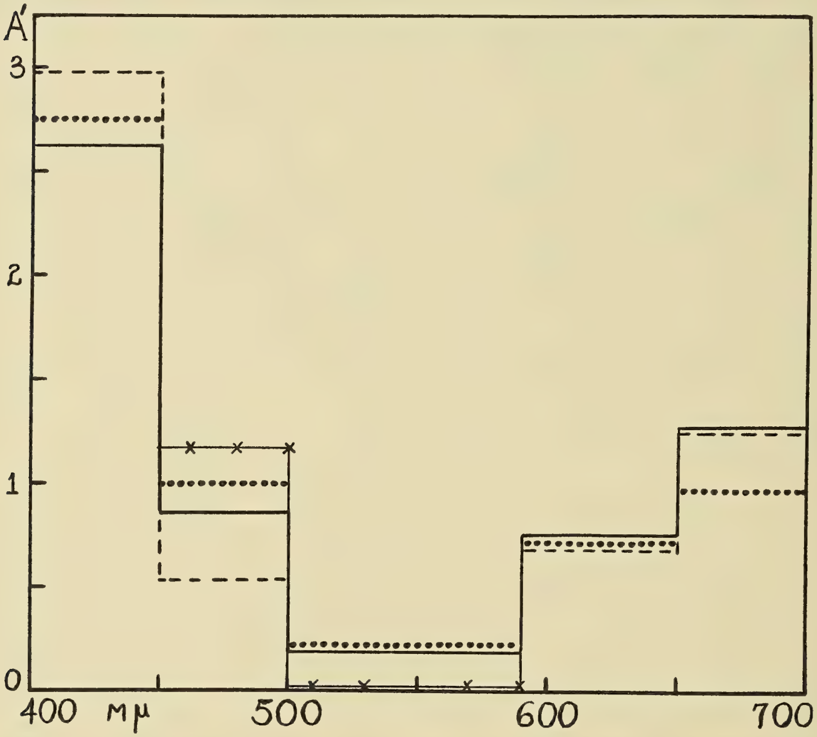

Fig. 3. The relative energy absorption spectrum of the electromotive system in *Valonia* in the visible range (solid line). The lines marked by crosses are the alternative values calculated for filter no. 12. The dashed line is the relative absorption for the chlorophyll mixture $a/b = 3$; the dotted line is for the mixture $a/b = 1$.

in table 2. Columns 4 and 5 of table 3 give the relative absorption for the indicated chlorophyll mixtures calculated in the same way as for the filter transmission regions in table 2.

The A' values for the five spectral regions are plotted against wave length in millimicrons as the rectangular areas bounded by the solid line in figure 3. The light lines marked by crosses between 450 and 590 mμ are the values in parentheses for filter no. 12 in table 3. The dashed lines give the relative absorption for chlorophyll $a/b = 3$, the dotted lines the relative absorption for $a/b = 1$.

The writer would like to re-emphasize here that A' is a characteristic of the electromotive system. The general correspondence between the distribution of A' with wave length and the distribution of the relative absorption by chlorophyll is complete between 400 and 700 mμ. This is regarded as unequivocal proof that chlorophyll is the photosensitive material which determines the relation of light to the protoplasmic E.M.F. in *Valonia*. No other photosensitive material plays an appreciable part in the electromotive mechanism in the visible part of the spectrum. Even if the alternative values derived with filter no. 12 are accepted, the similarity in contour of the plots is decisive.

Although the method of arriving at the relative energy absorption spectrum is essentially quantitative, no inferences can be drawn from the *degree of correspondence* of A' with the relative absorption by chlorophyll. The correspondence is, on the whole, closer to the relative absorption for the mixture $a/b = 1$, as was true for the A' values for the filters in table 2. In view of the nature of the inherent errors of the method, however, any conclusions as to the composition of chlorophyll in *Valonia* from this result would be unjustified.

DISCUSSION

The Sources of Error

Aside from work on the visual system, no analysis similar to the present one has come to the writer's attention. Hecht has determined for *Mya* (1921) and *Pholas* (1928) the distribution with wave length of the reciprocal of the relative light intensity necessary to produce a constant response. This function is equivalent to $Q_f I_f$ in equation (1) obtained under conditions making $Q_u I_u$ constant. Hecht considered the plot against wave length to be the absorption spectrum of the photosensitive substance. The absorption spectrum for photokinesis of *Unionicola* as determined by Waterman (1937) is a plot of I_u against wave length with $Q_f I_f$ constant. Neither Waterman nor Hecht corrected his data for the quantum effect. For the human eye the reciprocal of the incident quanta necessary to yield a constant brightness sensation has been shown to correspond closely to the absorption spectrum of rhodopsin (Wald, 1938). This appears to be the only instance other than the present one for chlorophyll where the absorption spectrum of a substance determined from measurements of biological function has been compared quantitatively with the physically determined absorption spectrum.

The literature contains many studies of the effectiveness of different spectral regions upon the magnitude of biological processes wherein both interest and importance would attach to an analysis of the absorption spectrum of the photosensitive material. The possibility of the extension of the method to other systems is, in part, the excuse for examining in detail the inherent errors. No quantitative estimate of the magnitude of error is possible in the present work, but the importance of the error for the final result may be judged from its direction.

The sources of error may be classified as follows:

A. Errors in the conditions of illumination:

1. Variations of energy distribution of the source with voltage. In the majority of the experiments the voltage drifts were relatively slow, and the error in the use of the quantum areas for 2900° K is largely compensated by its presence in both numerator and denominator of the quantum area ratio. This was not always the case; for example, in the determination for filter no. 53 the voltage for white light was less than that for the filter. Q_u in consequence was too large, and, in part, this accounts for the large A' value.

2. Energy losses due to reflectance from the surfaces of the *Valonia,* the containing vessel, or the filters, for which no correction was available. The errors due to the first two factors are probably unimportant, for they appear with both white and filtered light, although the reflectance would be expected to vary with wave length. Reflectance from the filter faces is a systematic error entering into all the determinations. It cannot account for the fact that the filter A' values all tend to be too large. The error is in the opposite direction, for the product $Q_f I_f$ in equation (1) is too large by the factor of loss.

3. Screening effect of other pigments and absorbing systems. The effect of absorption by systems other than that responsible for the change in E.M.F. is to decrease the actual quantity of radiation incident on the latter. If the combined spectrum of all such systems is distributed more or less uniformly over the entire range under examination, the error introduced is a compensated one, and negligible. If the screening is principally confined to one end of the spectral range, its effect is to make the determined values at the other end of the range too great. It is evident from figure 3 that the total energy absorbed by any hypothetical screening substances in *Valonia* is too small in comparison with that absorbed by chlorophyll to deform the curve significantly. Since the analysis is independent of the actual transmission of the material, the screening substances must lie between the source and the photosensitive system to be effective, and can produce a serious error only in a bulky organ or tissue.

B. Errors inherent in the characteristics of the absorbing system:

1. Failure of the E.M.F. to attain a steady level. Because of the shape of the transmission curve of E.M.F. between steady levels (Marsh, 1939), a premature judgment of the level will tend to fix the intensity at too low a value. Since it would appear for both filtered and white light, this error should show no consistent directional tendency.

2. Inaccuracy of determination of intensity ratios for low-transmission filters at the flat portion of the E.M.F. : log intensity curve. The error should be random; it has been discussed in the section on intensity ratios.

3. Variations in concentration or state of chlorophyll in individual cells. The effect of such variations, which probably exist, should be random.

4. Effect of temperature. It has been shown (Marsh, 1936, 1937) that in the dark or in weak light (about 20 foot-candles) the E.M.F. has a Q_{10} of the order of 1.6 between 26 and 36° C, whereas at light intensities of the order of 200 foot-candles the temperature coefficient is of the order of 1. Presumably, between 20 and 100 foot-candles some temperature effect persists. The high infrared absorption at intensities necessary to match white light with the low-transmission filters always produced a rise in temperature in the containing vessel. This would increase the E.M.F. above the white-light level and make the intensities of match for the filters too small and the A' values too large. This error probably accounts for the deviation of the A' values in table 2 and its relation to transmission of the filters. The expected correspondence between the magnitude of deviation and the average unfiltered light intensity does not hold for the filter series, nor for individual values for any filter; it does hold for the five low-transmission filters.

C. Errors inherent in the analysis:

1. Assumptions concerning the characteristics of the tungsten lamp. Errors introduced by accepting the manufacturer's rating of total output as a basis for intensity calculations are completely compensated in the intensity ratios. Errors due to assignment of black-body temperature (Brackett, 1936, pp. 180–184) would similarly be made negligible in the quantum area ratios.

2. It is probable that the filter transmissions did not correspond precisely to catalogue specifications.

3. Errors due to the graphical summation of curve areas are unimportant.

4. Errors due to arbitrarily restricting the spectral region analyzed. Zscheile (1934) presented no data for wave lengths shorter than 395 mμ, at which point the absorption coefficients were moderately high, but decreasing rapidly. Winterstein and Stein (1933) determined the absorption spectrum of both components in benzene; while a close comparison of their results with those of Zscheile cannot be made owing to solvent differences and questions of purity of their materials, their curves show that an appreciable absorption occurs between 300 and 400 mμ. If we assign to the absorption coefficient the improbably large value of 30 for both components over this range, the relative absorption values in figure 3 are too large by about 6 per cent. Inclusion of the band 300–400 mμ in the energy distribution for the source would make Q_u, and consequently A', about 2.5 per cent greater. (This figure would be smaller if corrected for the screening effect of the lamp wall and the containing vessel.) Neither correction would be significant for the final result, and both corrections are certainly too large. The energy absorbed between 380 and 400 mμ is evidently too small a fraction of the total to affect the results with filter no. 2. The

absorption of light by chlorophyll above 700 mμ is negligible. If any other photosensitive material contributed to the effect of light above 700 mμ, the short-wave cut-off filter set should have yielded A' values growing progressively greater than the relative absorption with increasing wave length.

It would appear that the errors listed in A (save for A3) and C, which could be eliminated entirely by use of the appropriate measuring devices, are of less importance in producing the deviations of A' from the relative absorption than are the errors in section B. These latter could likewise be eliminated or reduced by a more refined technique and a greater number of measurements for each filter. Some degree of mutual compensation of the errors occurs in the final result, and the method tends to distribute the error over all, or a large part of, the spectrum. It may be concluded that, with moderate care, the functional determination of an absorption spectrum can be made with sufficient precision to enable the identification of the absorbing substance or to predict its absorption characteristics.

The Electromotive System

In an earlier paper (Marsh, 1936) it was pointed out that in any theory of bioelectric potentials which assumes the electromotively active materials to be inorganic ions of the cell or medium, the explanation of alteration of E.M.F. due to environmental agents which do not affect the ionic composition of the medium must be based upon changes in permeability of plasma membranes. It was emphasized that no general principle relates the direction of change in permeability with the direction of change of E.M.F. induced by the agents known to influence both properties. Visible light is one such agent. A definite connection exists between increase of permeability and light intensity (Lepeschin, 1930; Gellhorn, 1929); this relation is of the same nature for electrolytes and non-electrolytes. For the green plants and for visual receptor systems (Hartline, 1927–1928) an equally definite connection exists between the E.M.F. generated and incident light intensity. The two phenomena are quantitatively dissimilar, although no published demonstration has been made for the same material. On the basis of the relative effectiveness of different spectral regions on the magnitude of the two processes, it can be shown that the effect of light on the inherent E.M.F. of green plants (as exemplified by *Valonia*) is *completely independent* of the effect of light on permeability.

Brooks (1926) studied the influence of wave length of light upon the penetration of 2,–6,– dibromo phenol indophenol into the sap vacuole of *Valonia macrophysa* (a near relative of *V. ventricosa*). The cells were exposed to blue sky light transmitted through Corning glass filters, and the rate of penetration was determined by measuring the concentration of dye in the sap at intervals over a period of ten hours. The decreasing order of effectiveness of light in increasing the permeability of the cells to the dye as measured by the rate of penetration was: white, ultraviolet, blue, green, red, dark. The effectiveness was plainly less dependent upon the total incident energy than upon the wave length; the ultraviolet filter had the lowest energy transmission. While an absorption spectrum of the photosensitive material responsible for the permeability increase with light cannot be constructed from Brooks' data, such a spectrum would apparently increase steadily with decreasing wave length from the red to a maximum in the ultraviolet. At all events it bears no resemblance to the absorption spectrum for E.M.F. shown in figure 3 of this paper. Essentially the same results were obtained by Lepeschin (1930) on the effect of wave length on the penetration of methylene blue into *Elodea* leaves. That the "effectiveness spectrum" for permeability possesses something like general application is shown by the work of Packard (1925), who demonstrated a strikingly similar relation for the penetration of NH_4OH into *Paramecium*.

The significance of these results is obvious. Had chlorophyll been the absorbing substance in Brooks' experiments, the order of effectiveness of the filters would have been quite different. Had the effect of light in the present experiments been due to alteration of the permeability of plasma membranes to ions of the medium (such as potassium), the energy absorption spectrum should have resembled that predicted by Brooks' data. The absorption of the light by chlorophyll is considered by the writer to be proof that the electromotively active materials responsible for the production of electrical energy are created in the complex of metabolic activities associated with photosynthesis. Brooks' results deny the existence of any indirect connection between photosynthesis and cell permeability.

It was shown in the first paper of this series that the relation of the inherent E.M.F. in *Valonia* to light intensity is consistent with the interpretation that photosynthetically released oxygen is the immediate determining factor, and that this fulfills a specific prediction of Lund's oxidation-reduction theory of the origin of bioelectric potentials. The present demonstration that chlorophyll is the photosensitive material which absorbs the radiant energy as a preliminary to its appearance in electrical form satisfies a necessary condition for the application of the oxidation-reduction theory. Within the past few years there has appeared an increasing recognition of the intimate part played by metabolic processes in the establishment and maintenance of bioelectric potentials (e.g., Blinks, 1937; Francis and Gatty, 1938), a fact which had long been ignored. The rational and far-reaching connection between E.M.F. and metabolism expressed by the oxidation-reduction theory has been rejected by many workers because of their unwillingness to accept the implication that some cell structure behaves as a reversible electron acceptor. In its place have been offered two hypotheses which appear to the writer still less satisfactory: (1) It is suggested that metabolism may control the properties of the membrane across which modified diffusion potentials become established. The nature of the control is not stated; in view of the lack of any correspondence between permeability and E.M.F., discussed at the beginning of this section, it promises to remain nebulous. The effect of the membrane in controlling the mobility of any ionic species is a permeability relation which should be demonstrable. The distinction between the effect of wave length on permeability, as shown by the work of Brooks and others, and the effect of wave length on the inherent E.M.F. of

Valonia constitutes a clear demonstration of the complete absence of the very type of control of the properties of the limiting membrane demanded by the postulated relation. (2) The second hypothesis connecting metabolism and bioelectric potentials is that the metabolic processes create organic ions which then set up diffusion potentials at the plasma membrane. This suggestion, which raises more problems than it settles, will be discussed in a later contribution.

SUMMARY

1. The steady E.M.F. of impaled *Valonia ventricosa* illuminated with light of limited spectral composition was matched with white light.

2. The ratio of the intensity of white to that of filtered light for an E.M.F. match was independent of the magnitude of the E.M.F. matched and of the absolute intensities.

3. From the known spectral energy distribution of the tungsten lamp employed as a light source, the known spectral transmission of the filters, and the determined intensity ratios, the relative energy absorption of the electromotive system was calculated for the transmission ranges of the filters. The analysis assumes that an E.M.F. match will occur for filtered and white light when the number of quanta absorbed per unit time is equal.

4. The relative energy absorption is shown to compare fairly well for the filter series with the relative absorption of chlorophyll mixtures over the same spectral range calculated from the determinations of the absorption coefficient published by Zscheile.

5. From the data for four of the filters it is possible to divide the spectrum into five regions: 400–450, 450–500, 500–590, 590–650, and 650–700 mμ. These regions approximately isolate the four main absorption peaks and the absorption minimum for the two chlorophyll components.

6. A relative energy absorption spectrum is constructed over the range 400–700 mμ which is similar in form to the corresponding relative absorption spectrum for chlorophyll.

7. It is concluded that chlorophyll is the photosensitive material absorbing the radiant energy responsible for the effect of light upon the protoplasmic E.M.F. in *Valonia*. The chlorophyll system is, therefore, an intimate part of the electromotive mechanism.

8. The errors inherent in the method are discussed.

9. It is pointed out that the effect of light upon the E.M.F. is independent of its effect upon the permeability of the plasma membranes, even through some vague, secondary control exerted upon membrane properties through metabolism (photosynthesis).

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VII

The Chromatophore System of the Larvae of Crangon armillatus

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With one plate

[ISSUED MAY 16, 1940]

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The Chromatophore System of the Larvae of Crangon armillatus[1]

INTRODUCTION

The ability of certain animals to imitate the color in their background is so striking that it has received the attention of investigators for a considerable period of time. This feature has been observed principally in fishes, amphibians, and reptiles of the vertebrates, and in crustaceans of the invertebrates. Both the color mechanisms and the systems within the animals responsible for controlling these mechanisms have been studied. In recent years most studies have been directed toward discovery of the system responsible for the control of the color bodies. The view usually given as regards crustaceans is that a neurohumor borne in the blood of these animals controls the chromatophore system. A review of the work done with crustaceans is given by Parker, Brown, and Odiorne (1935). It is the writer's intention in this paper to add to this field of study the results of his observations on the reactions of the chromatophore system of the larvae of *Crangon armillatus.*

These larvae were obtained by keeping the egg-bearing females in finger bowls until their eggs hatched. The larvae were then transferred to other finger bowls and kept until they were studied. On hatching, each larva possesses a single pair of chromatophores in its tail at the base of the telson (plate 1, fig. 2). Within a few hours three more pairs of chromatophores appear on the larva: one pair at the base of the abdomen and two pairs on the cephalothorax (plate 1, fig. 1). Additional pairs of the pigment bodies of this animal develop within two or three days, so that they are visible on the antennae, eyes, cephalothorax, abdomen, and the tip of the telson. All chromatophores appearing in this larva are bright red in color and will be referred to henceforth as erythrophores. The whole body of a larva of *Crangon armillatus* is transparent except for the black eyes and the erythrophores. This transparency together with the small number of erythrophores make this animal suitable for a study of the chromatophore system. To follow the changes through which any particular erythrophore passed was quite convenient. The large pairs of these organs at the base of the abdomen and at the base of the telson were the ones usually observed in arriving at the data presented and discussed in this report.

Most of the larvae studied during the investigation reported herein were not more than one day old. A few older specimens with a greater number of erythrophores were subjected to the same experiments as were the younger ones to see whether there was any difference in the reactions of the erythrophores of the two groups. In every instance the color bodies of the older larvae behaved precisely in the same manner as did those of the younger animals. Normal larvae and enucleated ones were subjected to the same experiments and in most instances were kept together in the same bowl during the experiments, though the enucleated specimens were allowed to overcome the shock of the operation by being kept in separate finger bowls several hours before they were subjected to this experimentation.

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[1] The writer is deeply grateful to Carnegie Institution of Washington for making it possible to carry on this problem at the Tortugas Laboratory.

The majority of the erythrophores of a larva kept in a clear finger bowl on a light-colored table in daylight disperse fully. This dispersion continued while the animals were examined through a microscope using transmitted light. A few of these organs, however, remain in the concentrated condition. These two opposing reactions of the erythrophores in a single animal are shown in plate 1, figure 1.

Experiments and Results

Both normal specimens and enucleated ones were placed in illuminated white finger bowls. Within a few minutes the erythrophores of the uninjured larvae became dispersed if they had previously been in a concentrated state, or they remained dispersed, while the erythrophores of the enucleated specimens became concentrated. This concentration in the erythrophores of the enucleated specimens required a considerably longer time than did the dispersion of the pigment of the normal larvae. During the study of these color bodies, it was noticed that the pigment in addition to migrating seemed to disappear as if being dissolved, though it reappeared during the dispersion phase. In its completely concentrated condition the erythrophore always disappeared entirely, as is shown in plate 1, figure 4.

In order to study further the reactions of the chromatophore system of these larvae, both the uninjured specimens and enucleated ones were kept in illuminated black finger bowls. The erythrophores of the normal specimens faded out completely within a few hours. This fading was gradual, leaving a few granules of pigment in the processes of the erythrophores until just before complete disappearance (plate 1, fig. 3). This blanched condition persisted as long as the animals were kept in the illuminated black bowls. In contrast to the erythrophores of the uninjured specimens, these organs of the enucleated larvae did not react in a uniform manner, though the majority of them became dispersed when these animals were kept in the illuminated black bowls. It must be stated here that the dispersion of pigment in the enucleated animals in the black bowls was not as complete or as uniform as was the concentration of pigment in the enucleated specimens which were kept in the illuminated white bowls.

Both normal and enucleated specimens were kept in finger bowls in a dark room for several hours. The erythrophores of both the normal and the enucleated larvae thus treated became dispersed maximally (plate 1, fig. 2), though the dispersion of the pigment of the color bodies of the enucleated larvae was not as complete as that of the normal animals. The dispersion in both specimens took place within a few minutes.

Discussion

To review briefly the results given in the previous section of this paper in regard to the behavior of color bodies of normal larvae, the pigment of the erythrophores of these larvae became dispersed in response to white background and concentrated in response to black background. These responses are exactly opposite to those of most crustaceans when they are subjected to white and to black backgrounds. In most crustaceans the dark pigment bodies become concentrated in response to white background and dispersed in response to black background. This view is given by Brown (1935*a*), who reports that the red pigment of *Palaemonetes* is concentrated while this animal is subjected to white background and is dispersed broadly when the animal is kept over black background. Exactly the same response is given by the red pigment of the red cancroid crab of Bermuda, according to Abramowitz (1935).

The reactions of the erythrophores of enucleated *Crangon* larvae do not conform strictly to the usual behavior pattern of the chromatophores of most enucleated crustaceans. In the majority of crustaceans that have been deprived of their eyes, the dark pigment bodies become dispersed and remain in this condition regardless of their background (Parker, Brown, and Odiorne, 1935, p. 448). The erythrophores of the enucleated *Crangon* larvae observed in the present experiments did respond to different backgrounds. The concentration (fading) of pigment while the animals were over the white background was particularly noticeable. The dispersion of pigment while they were over the black background was not uniform, comparing these reactions in all specimens thus treated. It should be pointed out here that the responses of the erythrophores of the enucleated larvae are the same as the responses of the dark chromatophores of most normal crustaceans.

The writer interprets the results of his experiments with the larvae of *Crangon armillatus* as showing that their chromatophore system acts as an aid to their sensory system. These larvae are very responsive to light stimulation, always moving instantly toward the light source whether it be weak or strong. In high lights obtained over white background, the erythrophores become dispersed, thus protecting the sense organs. In low light intensities resulting from a black background, these organs fade in color and thus permit a greater amount of light to fall on the sense organs. This interpretation would imply, then, that the nervous system of this larva does play some part in the control of its chromatophore system. This view is in part in agreement with Brown (1935*b*), who states that hormones which concentrate the red and yellow pigments of *Palaemonetes* are formed in the eyestalks and the central nervous organs. Unfortunately the writer could not do any injection of eyestalk materials because of the microscopic size of the larvae of *Crangon*. The data do show, however, that the ablation of the eyes of these larvae removes the chief sensory organs and thus permits the erythrophores to react independently and directly to stimulations producd by different backgrounds.

SUMMARY

1. The erythrophores of normal larvae of *Crangon armillatus* react as follows according to different backgrounds: (*a*) the pigment is dispersed when the animals are kept in a white illuminated bowl; (*b*) the pigment becomes concentrated completely when the specimens are subjected to illuminated black bowls; (*c*) the pigment is dispersed when the specimens are kept in total darkness.

2. The erythrophores of enucleated specimens show the following conditions when subjected to different backgrounds: (*a*) the pigment becomes concentrated when the specimens are kept over a white illuminated background; (*b*) the pigment is dispersed when these specimens are subjected to black illuminated bowls; (*c*) the pigment is dispersed in the specimens which are kept in total darkness.

3. The time required for concentration of pigment in the erythrophores is much longer than that required for its dispersion.

4. Ablation of the eyes permits the erythrophores to react directly to stimulations caused by different backgrounds.

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PLATE

Plate 1

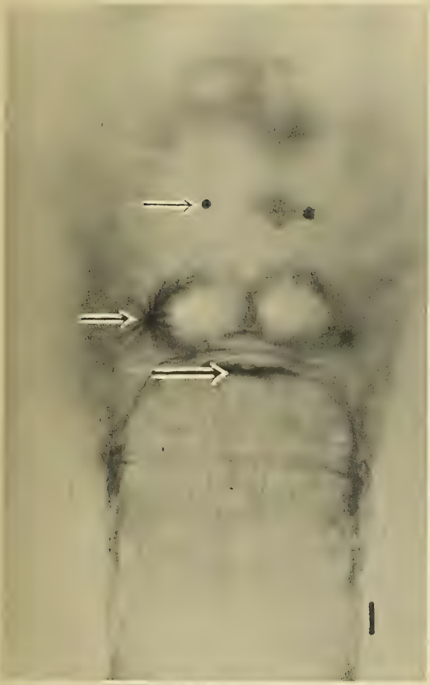
Photomicrographs, × approximately 300. Arrows indicate erythrophores.

Fig. 1. Two pairs of erythrophores on the cephalothorax and one pair on the base of the abdomen. Note the concentrated state of the upper pair on the cephalothorax, while the lower pair is in a dispersed state.

Fig. 2. The pair of erythrophores at the base of the telson in a fully dispersed state. This condition is obtained with normal specimens in clear bowls on a light table, in white illuminated bowls, and in total darkness, and with enucleated specimens in black illuminated bowls and in total darkness.

Fig. 3. Intermediate phase of the erythrophores at the base of the telson. The erythrophores pass through this phase in concentration or in dispersion of the pigment.

Fig. 4. The arrow indicates the location of the concentrated erythrophores. Normal specimens over an illuminated black background and enucleated specimens over an illuminated white background show this condition.



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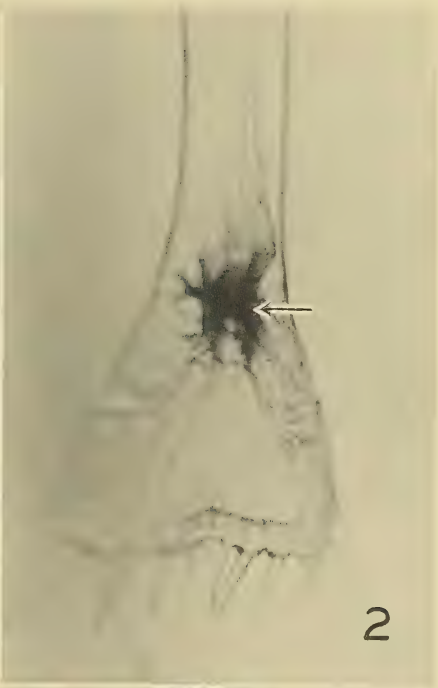

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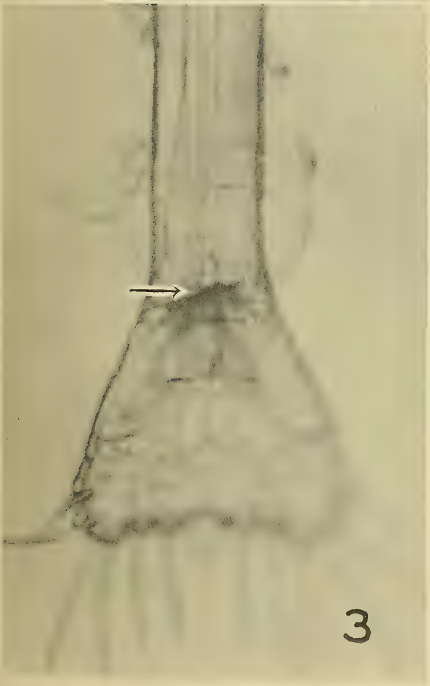

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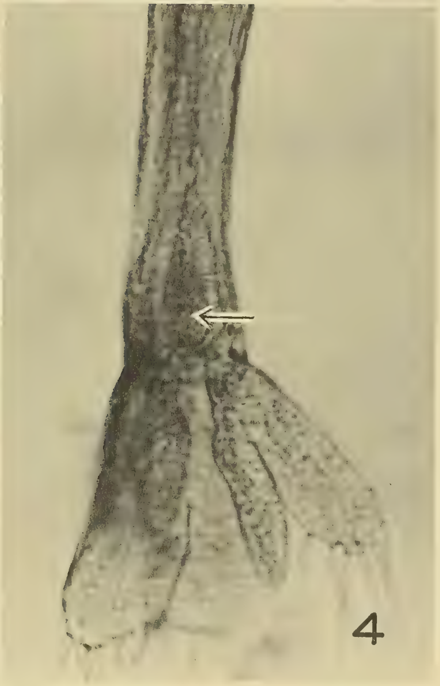

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PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

VIII

The Structure of Zooxanthellae

By William L. Doyle and Margot Metcalfe Doyle

Bryn Mawr College

With thirty-seven text figures

[ISSUED MAY, 16, 1940]

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The Structure of Zooxanthellae

INTRODUCTION

The association between zooxanthellae and invertebrates is known to be of widespread occurrence (Buchner, 1930), and the nature of this symbiosis is widely different in the various host animals (Yonge, 1935). The experiments of Boschma, Smith, Yonge, and others have contributed much to our understanding of the physiology of the association in the various coral-reef organisms (Boschma, 1925, 1926; Smith, 1935, 1936, 1939; Yonge and Nicholls, 1931*a*, 1931*b;* Yonge, Yonge, and Nicholls, 1932; Yonge, 1936). The relative abundance of the organisms in relation to light conditions in the corals has been pointed out by Gardiner (1931). The structure of the zooxanthellae has been dealt with, largely from fixed preparations, by Brandt (1883), Yonge and Nicholls, and Smith (*loc. cit.*).

In an investigation of the nature of the association between zooxanthellae and the foraminiferan *Orbitolites duplex,* one of the present authors noted that a considerable variation in the cytological structure of the zooxanthellae occurred under various experimental conditions (Doyle, 1934, 1935). Since these variations were of a qualitative as well as of a quantitative nature, and since certain details of the structure as found by Yonge and Nicholls and by Smith (*loc. cit.*) could not be confirmed, the structure of the zooxanthellae in various reef organisms under various conditions was investigated. The observations made on foraminifera and corals in 1934 and 1935 were rechecked and supplemented in 1939.

The forms investigated include *Orbicella, Acropora, Siderastrea, Meandrina, Platygyra, Porites, Porpita, Cassiopea, Folliculina,* and *Orbitolites.* Zooxanthellae from each of these genera were studied in the living condition as well as after fixation. They were prepared for study in the living condition in various ways. In the foraminifera, the shell was either crushed with needles or dissolved away using increased CO_2 in the sea water (see below). In the corals, small pieces were chipped off and the bits of stone teased out of the preparation to permit examination with the 2-mm. lens.

GENERAL STRUCTURE

Under appropriate conditions zooxanthellae contain the following structures: a plastid of reticulate structure having embedded in it a colorless pyrenoid surrounded by an amyloid (starch) layer, additional amyloid (starch) grains containing no pyrenoid, an "assimilation product," oil droplets, red pigment granules, crystals of calcium oxalate, and the regularly occurring nucleus, cell wall, and hyaline cytoplasm. Figure 1 indicates in outline the relation of these structures. Under certain conditions some of the structures may be entirely absent. The authors cannot agree with Yonge that the assimilation product is the only structure visible in the living zooxanthellae. With the exception of the nucleus and the plastid reticulum, which could but rarely be distinguished, all structures were clearly discernible with a 2-mm. oil immersion lens N.A. 1.25, and a 10 × ocular; it is, however, necessary to use a polarizing attachment in the study of the calcium oxalate crystals.

The organisms vary in size from 6 to 14 microns and have a cellulose wall as described by Yonge and Nicholls, which varies in prominence in different forms. In most corals the cell wall of the zooxanthellae is a well-defined structure which gives the clear-cut staining reactions characteristic of cellulose. In other forms it is sometimes more difficult to demonstrate the presence of cellulose in the wall. However, if the material is extracted with water followed by alcohol for a length of time sufficient to remove the pectins and pigment, good staining reactions can be obtained with chlorzinc iodide and iodine sulphuric acid reagents. The cellulose appears to vary in thickness in individuals and is thinnest in those specimens which are growing most rapidly.

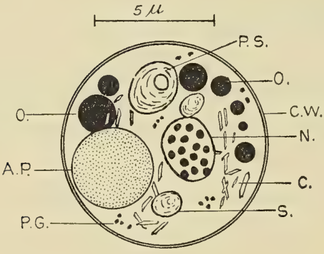

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Fig. 1. Structure of a typical zooxanthella. Composite drawing.
A.P., assimilation product; *C.*, calcium oxalate crystals; *C.W.*, cell wall; *N.*, nucleus; *O.*, oil; *P.G.*, pigment granules; *P.S.*, primary starch granule; *S.*, secondary starch grain.

The nucleus, as stated above, is seen only with difficulty in the living organism. It is readily identified by use of methyl green in 1 per cent acetic acid. With aceto-carmine the results are poor owing to the swelling of the oil droplets and the formation of a crystalline precipitate. The chromosomes of the resting nucleus in the fixed condition appear as granular masses (fig. 1).

The calcium oxalate crystals were studied between crossed Nicols and a first order red plate. Though not definitely identified as calcium oxalate by Brandt (1883), they were clearly figured by him as occurring in the zooxanthellae. The crystals are insoluble in carbonic acid and acetic acid but are readily removed by mineral acids. From observation of their crystalline form and solubilities it is apparent that they are either mono- or trihydrate calcium oxalate.

The relative amount of crystals is very variable, as will be seen below, and they are extremely variable in quantity in zooxanthellae from different sources. Their occurrence is definitely dependent on light conditions, though this may be a secondary effect. Owing to the lack of uniformity of incident light intensities obtainable in corals—where the skeletal septa may act as screens to the zooxanthellae—more reliable experimental data concerning the factors involved are to be obtained by a study of the zooxanthellae from foraminifera, although the screening effect of the shell in this case may still destroy precision. Nevertheless it is apparent from the study of statistically large numbers of zooxanthellae from corals that the organisms from the more intensely illuminated regions of the coral in question contain fewer calcium oxalate crystals than those from the darker regions of the same coral.

The oil droplets in the living organism are found to be either reddish in color or colorless. The red pigmentation of the cells appears to be segregated at times in granules of irregular outline, while at other times no granules can be seen and the oil itself is colored. The oil droplets blacken very readily with osmic acid, and stain blue with Nile blue sulphate only after prolonged treatment with the dye.

In preliminary notes, Doyle (1934, 1935) has referred to the independence of the assimilation product and the plastid. He also described the plastid as "composed of a colorless central pyrenoid surrounded by an amyloid layer." It is clear that two distinct structures exist in the cytoplasm, but it is a mistake to term the pyrenoid-containing body a plastid. It is rather the principal or primary amyloid body, which is in turn embedded in a reticular plastid discernible with difficulty. The region surrounding the pyrenoid may become very deficient in amyloid material (fig. 13), in which case the region no longer stains purple with iodine as it does when a greater amount of amyloid material is present. It is proposed to refer to this body as the primary starch granule, despite the disappearance of amyloid material from it at certain times. There may be objection to this designation, but it seems simplest in view of the fact that it is to be distinguished from another structure which has been designated the "assimilation product." Obviously the latter and the primary starch granule are both products of assimilation. Yonge and Nicholls (1931*a*) describe the "assimilation product" as containing amyloid material and a pyrenoid. Since we find that two structures are being thus described, and since the first part of their description coincides with ours, it is perhaps simplest to retain the term assimilation product for this "large, refractile, spherical body with a diameter frequently almost half that of the entire cell." The independence of this assimilation product and the primary starch granule may be seen by examination of Brandt's figures.

We have found that the assimilation product varies in diameter from one-fifth to one-half the diameter of the cell. The primary starch grain is rarely larger than one-third the diameter of the cell. In the corals the assimilation product is usually the more prominent in the living condition. The presence of the pyrenoid within the primary starch grain can be discerned in both living and preserved material, whereas no such central region was found in the assimilation product.

We have found that while the assimilation product is resistant to the action of very dilute acetic acid and to stronger concentrations of acetic acid after treatment with chromates, in the fresh condition it disintegrates upon application of reagents and fixatives containing more than 2 per cent acetic acid.

This may be sufficient to account for Yonge and Nicholls' assumption that the assimilation product and the primary starch grain are identical, since they state that after "fixation with Bouin (sometimes also after fixation with Flemming), a central pyrenoid of about half the diameter of the assimilation product, 'lying within a clear area,'" is all that can be distinguished. They also find that "after treatment with dilute acetic acid followed by iodine it [the assimilation product] stains a dark brown with an underlying color of reddish violet." It would seem that since acetic acid removes the prominent refractile structure, the remaining primary starch granule, now the most prominent feature, was mistaken for the former.

The simplest way in which the structures in question may be definitely resolved is to place fresh material in a solution of methyl green in 1 per cent acetic acid. The chromatin granules are stained an emerald green, the assimilation product is stained a dirty purple, and the primary starch granule is unstained but readily distinguished by its central pyrenoid.

Prior to the division of a cell there may be two assimilation products in the same cell. There may also be two primary starch grains. Two of each of the structures has not yet been observed in one cell.

Zooxanthellae in the Foraminiferan Orbitolites duplex

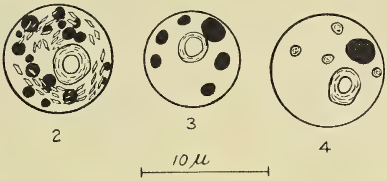
Orbitolites duplex, which contains many thousands of zooxanthellae, was obtained from the leaves of turtle grass, *Thalassia testudinum,* collected from the moat at Fort Jefferson and from Bird Key Reef. (*Thalassia testudinum* is the grass referred to as *Posidonia* by Cushman [1922]). Either the foraminifera were removed from the grass with a scalpel or the grass was left in large crystallizing dishes until the *Orbitolites* left it and attached themselves to the dish. The sea water was changed daily, using freshly collected water, since the laboratory supply proved toxic. It is interesting to note that the *Orbitolites* congregate at the light side of the dish.

The cytoplasm in the foraminiferan is not always evenly distributed throughout the shell. It may be less abundant in the outer chambers, or it may be unevenly clumped in various regions or to one side. Similarly, the zooxanthellae may be unevenly concentrated in the cytoplasm, never being so concentrated in the central chamber as in the peripheral regions. As found on the reef, the zooxanthellae average 8.1 microns in diameter. It is estimated that an *Orbitolites* 2 mm. in diameter contains on the average 16,000 zooxanthellae. In one specimen containing young megalospheres in the outer chambers, the zooxanthellae were counted in some of the megalospheres, the counts being 540, 470, 500.

The effects of light on the structure of the zooxanthellae were studied in a series of experiments conducted under the following conditions: in normal gas tensions; in increased carbon dioxide tension; in oxygen; in hydrogen.

The Effects of Light in Normal Gas Tensions

Experiment 7. Orbitolites were kept in the dark in normal sea water for 7 days. At the end of this time the zooxanthellae had the appearance shown in figure 2. One group was then returned to normal light on the laboratory table. At the end of 3 days the starch content was unchanged (fig. 3), the oil had decreased in amount, and the calcium oxalate had also decreased in amount but was still present (not drawn). At the end of 5 days a slight increase in starch was noted, the oil had decreased until only one droplet was left (fig. 4), and the calcium oxalate was still further reduced in amount (not shown). In those kept in the dark for the corresponding times, the calcium oxalate increased somewhat, and the oil and the starch decreased. Since the experimentally produced changes in structure, even over comparatively long periods of time, were small in relation to the individual variations among the zooxanthellae, this type of experiment was deemed inconclusive and the procedure abandoned.



FIGS. 2–4. Effect of light on zooxanthellae from *Orbitolites*.
Fig. 2, after 7 days dark.
Fig. 3, after 7 days dark and 3 days light.
Fig. 4, after 7 days dark and 5 days light.

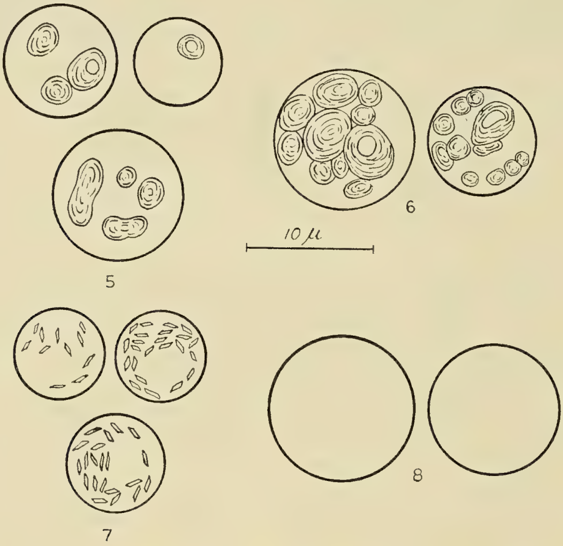
For explanation of structures drawn in all text figures, see figure 1. Figures 2–37 are from camera lucida drawings and have been selected from several in each instance in an attempt to show the full range of variation in each experiment. The drawings were made from the living organisms in most cases. Calcium oxalate was distinguished between crossed Nicol prisms and a first order red plate. Identification of starch was confirmed by staining with dilute iodine solution.

The Effects of Light in Increased Carbon Dioxide Tensions

Experiment 8. Twenty to 50 specimens of *Orbitolites* were put into 150 ml. of sea water in an Erlenmeyer flask and CO_2 bubbled through at a rate of 40 to 200 ml. per hour. Specimens kept in the light were subject to diffuse daylight which reached a maximum of about 800 foot-candles. It is of interest to note that the calcareous parts of the foraminiferan shell are dissolved in sea water under increased CO_2 tension, and that if the gas tension is adjusted so that it requires 48 hours to remove the carbonate from the shell, the foraminifera are uninjured and in 10 to 14 days after removal from sea water acidulated in this manner and return to normal sea water one may observe the formation of 1 to 2 rows of newly laid-down shell. The new shell is formed both in normal light and in darkness. Figures 5 and 7 show the relative amount of starch and calcium oxalate present in specimens kept in normal sea water under normal light conditions. Specimens kept under the same light conditions but with increased CO_2 tension showed a marked increase in starch and a total disappearance of calcium oxalate 24 hours later (figs. 6, 8).

Experiment 12. A gas train was set up constructed of Erlenmeyer flasks each containing 50 organisms. The entering gas bubbled through and the escaping gas entered the next flask. In this experiment, flasks 2 and 5 were wrapped in black paper to exclude all light. The others were exposed to the diffuse light from a north window. Flasks 1, 2, and 3 had CO_2 bubbled through them; flasks 4 and 5, air.

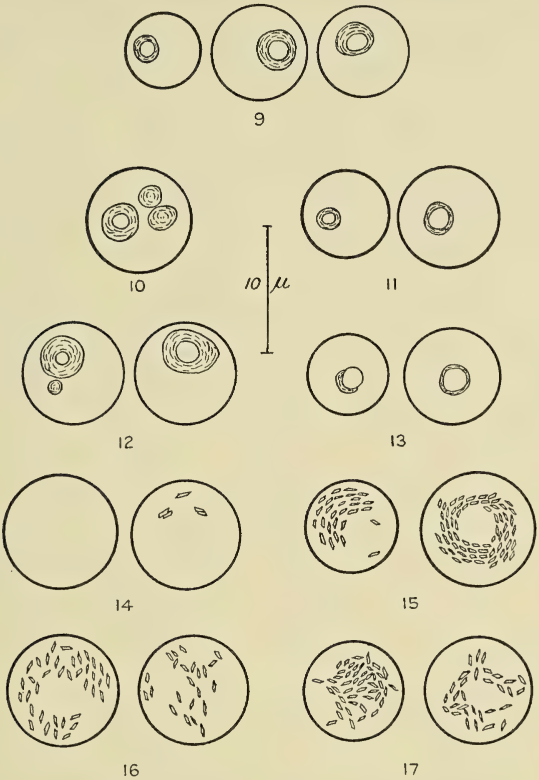
The condition of the zooxanthellae at the start of the experiment (June 25, 7:30 a.m.) is shown in figure 9. The average condition after 10 hours (June 25, 5:30 p.m.) of normal daylight in flasks 1 and 3 (CO_2) and 4 (air), and of darkness in flasks 2 (CO_2) and 5 (air), is shown in figures 10 to 17. From the figures it will be seen that the starch has decreased most in those specimens kept in the dark with air bubbling through (fig. 13). A slight increase in starch is to be noted in the specimens in flasks 1 and 3 (CO_2 and light, fig. 10). It is much more noticeable however, that the calcium oxalate has almost disappeared from specimens in flasks 1 and 3 (CO_2 and light, fig. 14). That this is not due to altered pH is shown by the abundance of calcium oxalate when light is excluded (flask 2, CO_2 and dark, fig. 15). The high CO_2 tension and resulting acidity in experiment 12 is not consistent with good growth of the zooxanthellae, and consequently only relatively short-time experiments could be run.



Figs. 5–8. Effect of increased CO_2 tension on zooxanthellae from *Orbitolites.*
Fig. 5, starch content in normal sea water and light.
Fig. 6, increased starch content in increased CO_2 and light.
Fig. 7, calcium oxalate content in normal sea water and light.
Fig. 8, decreased calcium oxalate content in increased CO_2 and light.

The Effects of Light on Specimens in Oxygen

Experiment 13. In this experiment a gas train was set up, in which flasks 1 and 2 had O_2 bubbled through at a rate of 40 ml. per hour, while flasks 3 and 4 were kept as air controls. Flasks 2 and 4 were darkened and 1 and 3 exposed to light. Each flask contained 30 organisms. The experiment was set up on June 28, at 2 p.m., when the condition of the zooxanthellae was as shown in figure 18. Fifty-five hours later (June 30, 9 p.m.) the following changes had taken place: there was a disappearance of calcium oxalate from flask 1 (O_2 and light, fig. 23) and marked starch production in 1 and 3 (light, figs. 19, 21); some decrease in starch in 2 and 4 (dark, figs. 20, 22) and little change in calcium oxalate in 2 (O_2 and dark, fig. 24), 3 (air and light, fig. 25), and 4 (air and dark, fig. 26). Thus the formation of starch is not influenced appreciably by increased O_2 tension.

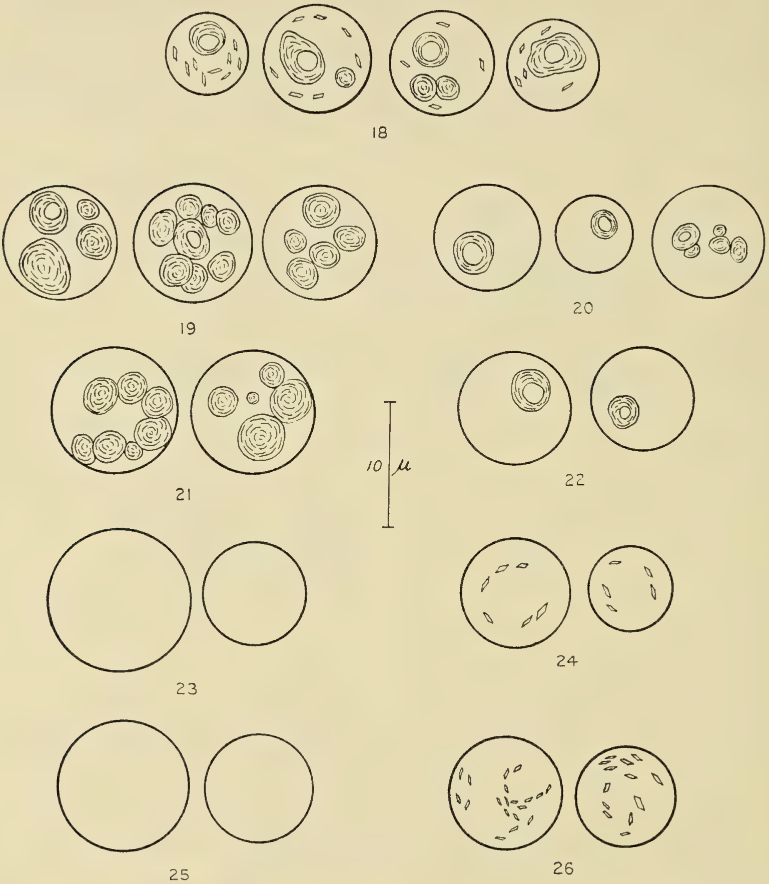


Figs. 9–17. Effect of increased CO_2 tension in light and dark.
Fig. 9, starch content at beginning of experiment.
Fig. 10, starch content after 10 hours increased CO_2 in light.
Fig. 11, starch content after 10 hours increased CO_2 in dark.
Fig. 12, starch content after 10 hours air in light.
Fig. 13, starch content after 10 hours air in dark.
Fig. 14, calcium oxalate content after 10 hours increased CO_2 in light.
Fig. 15, calcium oxalate content after 10 hours increased CO_2 in dark.
Fig. 16, calcium oxalate content after 10 hours air in light.
Fig. 17, calcium oxalate content after 10 hours air in dark.

The Effects of Light on Specimens in Hydrogen

Experiment 14. In this experiment the tensions of both O_2 and CO_2 in the medium were lowered by sweeping the fluid with H_2 in flasks 1 and 2, while 3 and 4 remained as the air controls. Each flask contained 40 organisms, the experiment was run from

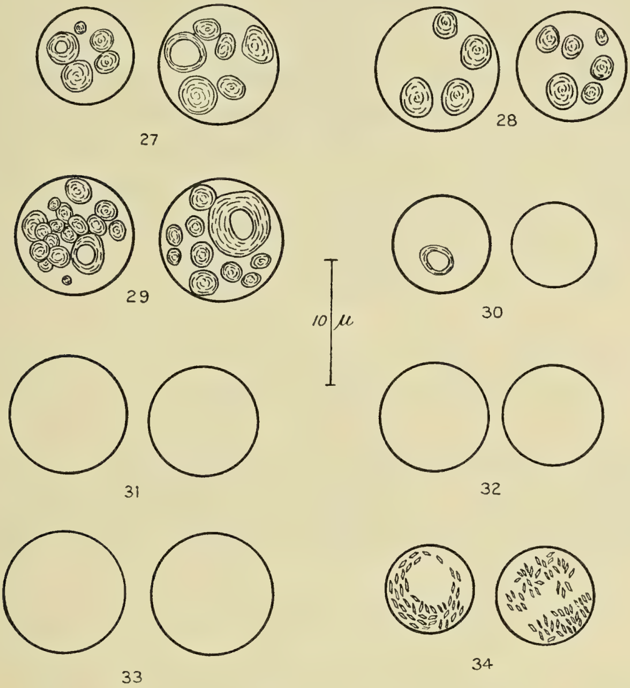
July 1, 8:30 a.m., to July 2, 7:30 a.m., and the results are shown in figures 27 to 34. Regardless of light intensity (with flasks 1 and 3 light and 2 and 4 dark), the specimens in H_2 showed no change in starch or calcium oxalate content (figs. 27, 28, 31, 32), whereas the controls in light showed some starch production and a diminution of calcium oxalate (figs. 29, 33), and the dark controls showed calcium oxalate production and starch reduction (figs. 34, 30). The organisms were not injured by the H_2.



Figs. 18–26. Effect of increased O_2 tension in light and dark.
Fig. 18, starch and calcium oxalate content at beginning of experiment.
Fig. 19, starch content after 55 hours O_2 in light.
Fig. 20, starch content after 55 hours O_2 in dark.
Fig. 21, starch content after 55 hours air in light.
Fig. 22, starch content after 55 hours air in dark.
Fig. 23, calcium oxalate content after 55 hours O_2 in light.
Fig. 24, calcium oxalate content after 55 hours O_2 in dark.
Fig. 25, calcium oxalate content after 55 hours air in light.
Fig. 26, calcium oxalate content after 55 hours air in dark.

The foregoing experiments demonstrate that in zooxanthellae from foraminifera the amount of starch present is markedly affected by the CO_2 tension in the sea water surrounding the foraminifera. Correlated with the changes in starch content are changes in the content of calcium oxalate crystals. The crystals become more abundant in the dark and tend to disappear when starch is being formed.

In the cytoplasm of the foraminiferan are certain constituents which appear to be identical with those in the zooxanthellae, namely, the starch grains, the calcium oxalate crystals, and the pigmented oil droplets. It may be argued that the presence of these structures in both protozoan and algal cells is due to identical synthesis by both cells, but it was found that increases in the relative abundance of these structures in the cytoplasm of the foraminiferan occur *only* when the zooxanthellae which it harbors are *distended with that type* of cytoplasmic inclusion. Thus zooxanthellae in the light with increased CO_2 tension may be twice the normal diameter. In foraminifera containing such zooxanthellae one finds many starch grains in the cytoplasm. How the materials are



Figs. 27–34. Effect of increased H_2 tension in light and dark.
Fig. 27, starch content after 23 hours H_2 in light.
Fig. 28, starch content after 23 hours H_2 in dark.
Fig. 29, starch content after 23 hours air in light.
Fig. 30, starch content after 23 hours air in dark.
Fig. 31, calcium oxalate content after 23 hours H_2 in light.
Fig. 32, calcium oxalate content after 23 hours H_2 in dark.
Fig. 33, calcium oxalate content after 23 hours air in light.
Fig. 34, calcium oxalate content after 23 hours air in dark.

transported from the cytoplasm of the zooxanthellae to that of the foraminiferan has not been observed, but it is suggested that such "overstuffed" zooxanthellae disintegrate during division.

No such simple dependence of structure on experimental conditions was demonstrated for the other cytoplasmic inclusions. Nevertheless, examination of the cytological picture in the zooxanthellae taken from a number of species demonstrates certain significant correlations between the inclusions.

In zooxanthellae from *Orbitolites,* the assimilation product is difficult to distinguish from the oil droplets in the living condition, and when much starch or oil is present it may be obscured. In the suitably stained preparations the assimilation product is readily distinguishable, and its relative size in two typical specimens is shown in figure 35. Under all conditions observed by the authors, the assimilation product in zooxanthellae from *Orbitolites* is never so large as it is in zooxanthellae from corals. In *Orbitolites* it would seem that the size of the assimilation product is inversely related to the amount of starch present.

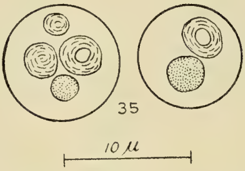


Fig. 35. Zooxanthellae from *Orbitolites*

Comparative Cytology of Zooxanthellae from Corals

It is difficult to maintain corals in the laboratory under controlled light conditions, consequently naturally occurring differences in light intensity were sought. A simple method for obtaining differences in light intensity is to select for comparison specimens from different depths. Another method is available for large heads of *Orbicella* and *Siderastrea,* since the total incident light reaching the upper surface of such forms is much greater than that reaching the sides. Furthermore, particular situations can be found in which the lower portions of such corals are screened from light reflected from the bottom and scattered light coming from the sides, by adjacent corals and beds of gorgonians.

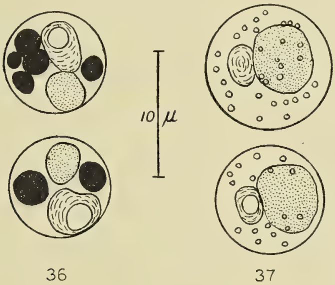
The conformations of the skeleton in the various genera of corals may also be expected to afford varying transmissions of the incident light to the surface of the zooxanthellae. How these transmissions are to be compared has not been determined.

The large coral *Orbicella acropora* was examined growing at different depths. The tops of large heads which are 1 to 1.5 feet below mean low tide level have a distinct yellow-green appearance, while the tops of the heads which are 8 to 12 feet below the surface are not nearly so light in color. This difference in shade which is apparent when the corals are viewed from a diving helmet is equally obvious when chips taken from shallow- and deep-growing heads are examined at the surface.

In any given coral head there is a marked variation in color over the surface of the coral. Thus, though the tops of different heads may vary in shade, the sides are more brownish and the most shaded portions are deep chocolate brown. Closer examination of the polyps growing in the lowest light intensities (in deep crevices, etc.) reveals that in the center of each polyp is a small but brilliant green area. This green coloration is unrelated to the zooxanthella coloration. The darkest specimen obtained was collected from a ledge of coral which was barely visible in a deep, well-shaded crevice (depth of ledge 15 feet below mean tide level and 1 foot from the bottom).

It is difficult to determine to what extent the variations in depth of color are due to differences in numbers of zooxanthellae in the tissues. *Acropora* (staghorn), however, is never as deeply colored as *Orbicella,* and has fewer zooxanthellae. The differences in hue are however directly related to the colors of the zooxanthellae.

Chips taken from the tops of lighter heads contain zooxanthellae in which the primary starch granule is present and contains amyloid matter staining purple with iodine, the assimilation product (less prominent), oil droplets in abundance but uncolored, no calcium oxalate crystals, and a few red pigment granules (in deeper-growing specimens). Chips taken from the more shaded portions of the same heads show a smaller primary starch granule in which the amyloid substance is not stained by iodine, a larger assimilation product, numerous red pigment granules, very few oil droplets all of which are red, and calcium oxalate crystals.



FIGS. 36, 37. Zooxanthellae from *Orbicella acropora.*
Fig. 36, from top of coral.
Fig. 37, from bottom of coral.

The reticulum of the plastid in the lighter portions is greener than in the darker portions, where it is yellow-brown. The exact tint is difficult to determine, since at all but the highest magnifications (1.5 mm., 1.3 N.A. objective) the reticulum is not discernible. Owing to the small size of the structures and the presence of small dark red granules in the light portions as opposed to larger lighter red oil droplets in the dark portions, it appears that it is the cytoplasm which bears the color.

Various other genera were examined and the conditions of their habitat noted in relation to the structure of the zooxanthellae.

A fairly large specimen of *Siderastrea radians* was examined and chips taken from it in different regions. In one chip taken from the head at a depth of 15 feet the zooxanthellae contained a primary starch granule, a large assimilation product, no pigment, and about 1 calcium oxalate crystal per 13 zooxanthellae. Another chip taken in a shaded place near the bottom at about 17 feet contained zooxanthellae having a single primary starch granule, a single assimilation product, a few pigmented oil droplets, and from 6 to 25 calcium oxalate crystals per zooxanthella.

A specimen of *Porites porites* was taken from approximately 12 feet below the surface of the water. The zooxanthellae examined from it were all found to contain numerous calcium oxalate crystals.

In *Meandrina sinuosa* which was kept in the dark for 41 hours after collection, a marked increase in the number of calcium oxalate crystals present occurred. In *M. meandrites* taken from 60 feet below the surface there were from 1 to 3 calcium oxalate crystals in about 5 per cent of the zooxanthellae observed. This coral contained the greatest density of zooxanthellae observed.

Platygyra viridis showed the largest primary starch granule found in the zooxanthellae of any coral observed. The calcium oxalate crystals were also more abundant than in zooxanthellae from most other corals.

The surface-living medusa *Porpita porpita* was found to contain numerous calcium oxalate crystals in the zooxanthellae contained in its tissues.

In the protozoan *Folliculina,* which is found on eelgrass and has a blue-green test which serves as a light filter, zooxanthellae are abundant in the cytoplasm. In the specimens examined calcium oxalate is abundant, and a single primary starch granule, oil droplets, and a small assimilation product are present.

In *Acropora muricata* treated with Lugols solution the assimilation product turns yellow to yellow-brown, the primary starch granule red-purple, and the secondary starch grains purple. When treated with osmic acid the oil turns black and the assimilation product greenish, and the starch remains uncolored. With Nile blue sulphate the assimilation product turns greenish blue and the starch remains unstained; the oil stains slowly in Nile blue sulphate in dilute acetic acid.

In the medusa *Cassiopea frondosa* zooxanthellae were found which contained a single primary starch granule, an assimilation product, oil droplets, pigment granules, and calcium oxalate crystals. With Nile blue sulphate, the starch and oil remained unstained while the assimilation product stained a bright greenish blue. In all *Cassiopea* examined the amount of calcium oxalate was higher than that found in corals, though not as high as in the foraminifera.

The above evidence is obviously fragmentary. It presents, however, materials from widely different sources all of which seem to the writers to conform to the conclusions reached in experimental work on *Orbitolites.*

Conclusions

In zooxanthellae from the various invertebrates studied for the present paper, calcium oxalate crystals are normal constituents of the cytoplasm. In all these forms the amount of calcium oxalate present is related to the amount of light reaching the cell. Natural variations in the amount of calcium oxalate in the zooxanthellae are clearly shown in the large heads of the coral *Orbicella acropora,* where zooxanthellae from the top of the head contain no crystals while those from the bottom of the head contain numerous crystals. Similarly, experiments carried out in the laboratory on *Orbitolites duplex* show that in the presence of light calcium oxalate tends to disappear, while in darkness it increases in amount. The converse is true for the amount of starch present.

There also seems to be some indication that the calcium oxalate crystals and the amount of starch present, which are inversely related in volume in *Orbitolites,* are also related in the maximum volume which they ever attain. Thus, starch is never very abundant in any circumstances in zooxanthellae from *Orbicella,* and neither is calcium oxalate; starch is sometimes very abundant in zooxanthellae from *Orbitolites* and so is calcium oxalate; *Platygyra viridis* has the largest primary starch granules found in the zooxanthellae of any coral examined and also a greater abundance of calcium oxalate crystals than is found in most corals.

The zooxanthellae in the corals, under the most intense light normally incident, present a cytological picture in which starch is never prominent but oil droplets are quite abundant. This supports the contention of Yonge (1934) that since the endoderm of the coral is so packed with zooxanthellae, there is never likely to be any superfluity of food for the zooxanthellae from which they can synthesize reserve materials. It seems probable to the authors that the zooxanthellae in all corals so far examined store reserve food at so slow a rate that the conditions under which the zooxanthellae in *Orbitolites* convey formed food materials to the host cell never obtain in the corals. The presence of numerous oil droplets in the cytoplasm seems not so much an index of a high rate of oil formation as an indication that the zooxanthellae are not capable of forming starch in any quantity.

Considering the whole picture, it is probable that for the greater part of the day the zooxanthellae as well as the corals are in need of oxygen. That some carbohydrate synthesis (and oxygen production) does occur is evident. It would appear not unlikely that the conversion of carbohydrates to fat during periods of oxygen lack accounts for the larger amounts of oil present in the more shaded regions.

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IX

The Structure and Composition of Valonia ventricosa

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With one text figure

[ISSUED SEPTEMBER 14, 1940]

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The Structure and Composition of Valonia ventricosa

INTRODUCTION

The physiology of *Valonia* has been dealt with extensively by numerous authors (see bibliography in Marsh, 1939; Steward, 1939; and Steward and Martin, 1937). The investigations of the relations of certain structural constants to the size of the cells and the effects of light on structure have been to some extent inadequate, and the experiments (carried out in the latter half of 1939) described in the present paper were designed to supplement them. Determinations were also made in order to obtain basic quantitative data suitable for an investigation of the distribution of enzymes between different cytological components of the cell. *Valonia ventricosa* was used throughout the investigation.

METHODS

Cells were collected from Long Key and the adjacent reef and brought to the laboratory, where they were kept in large glass jars of sea water which was changed daily. The cells were scraped free of adhering organisms and débris and kept in moderate light intensities (daily maxima 70 to 800 foot-candles). Small pieces of broken dead coral were kept in the jars with the *Valonia*.

An indication of the physiological state of the cells maintained under these conditions may be had from the following data on growth:

Cell no.	Weight (mg.)		Per cent increase in 21 days
	June 18	July 9	
71	10	17	70
72	85	139	63

THE RELATION BETWEEN CELL SIZE AND THICKNESS OF THE CYTOPLASMIC LAYER

The thickness of the cytoplasmic layer in *Valonia ventricosa* has been estimated at various values. Doyle (1935) stated that the protoplasmic layer was 5 to 8 microns thick, but failed to state that these values were obtained on cells 0.3 to 1.0 ml. in volume. The use of these values for the thickness of the cytoplasm has led to certain difficulties in explaining the accumulation of ions in the cytoplasm (Brooks, 1935). Accordingly the author has redetermined the thickness of the cytoplasm in cells of *V. ventricosa* obtained from the Tortugas region. The method of direct visual measurement of the thickness of the cytoplasm in intact cells is so difficult as to render the results inexact, and a different method was therefore employed. Coenocytes were measured and their volume and surface calculated from a consideration of weight, specific gravity, and dimensions. They were then punctured and placed in tapered centrifuge tubes containing sea water. They were allowed to stand for 15 to 20 minutes and then centrifuged at 2000 r.p.m. (12 cm. radius) for 45 minutes to one hour. During this process the cell moves to the centrifugal end of the tube and is laterally compressed so that some of the sap is extruded through the site of puncture and the cell wall becomes folded. At the end of centrifuging, the entire mass of protoplasm is to be found inside the cell wall at the centrifugal end of the sac formed by the cell wall. The cell wall and contents were then removed from the centrifuge tube by means of a pair of forceps and the lower portion cut off with a pair of scissors and dropped into a finger bowl of sea water. The protoplasm was found to form a compact mass lying on a segment of cell wall shaped like a watch crystal, from which it was easily removed with a pipette and glass needles and transferred to a 1-ml. graduated centrifuge tube. It was then centrifuged for 10 to 15 minutes and the volume estimated.

The accuracy of the method is much greater than may be obtained by direct microscopical measurement and is very precise for spherical cells. The largest error is introduced in the determination of the radii of spheroids which are markedly aspherical. Here, however, the volume measurement obtained by weighing serves as a check. The results given in table 1 are the most reliable estimates obtained.

TABLE 1. *Thickness of cytoplasm in relation to cell volume*

Cell no.	Volume of cell (ml.)	Area (sq. cm.)	Volume of cytoplasm (cu. mm.)	Thickness of cytoplasm (μ)	Estimated error (μ)
94	0.4	2.66	1.8	6.8	±0.3
110	2.6	10.2	9.5	9.3	±0.2
111	3.0	11.1	10.5	9.5	±0.2
109	3.2	11.9	11.3	9.5	±0.2
113	7.45	18.55	23.0	12.4	±0.04
93	14.5	32.0	50.0	16.0	±1.0
92	25.0	43.1	110.0	26.0	±2.0

With markedly aspherical cells it is very difficult to determine the surface area exactly, but in the case of cell 111 (fig. 1, *A*) the surface was calculated from a series of measurements of radii and from the volume and found to be 11.1 sq. cm. The cytoplasm in this cell was 9.5 ± 0.2 microns thick, which is very close to the values obtained for other cells of the same volume and surface.

For the purpose of ascertaining the volume of the cell, the changes in specific gravity of cells 1 to 6 ml. in volume are negligible (see below), so that one may take the weight as a basis of computation for the thickness of the cytoplasm. The results presented above were obtained from *V. ventricosa* directly from the reef and were checked with results obtained from cells kept in the laboratory at Tortugas and at Bryn Mawr for four months. Since these values show deviations of minor significance, it would seem that a fairly precise estimate of the volume and thickness of the cytoplasm may be obtained from the weight of the cell.

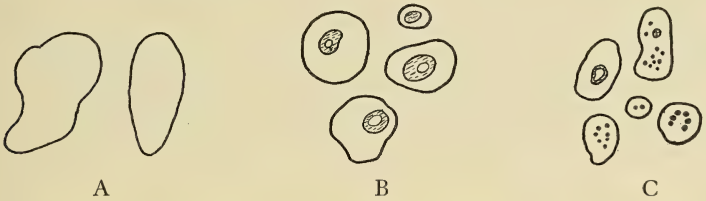

Fig. 1. *A,* two views of cell 111, showing its outlines (see text). *B,* plastids from cell kept in diffuse light, showing starch grains with central clear spaces. *C,* plastids from a similar cell kept in darkness for 14 days. Note the almost complete disappearance of starch and the presence of numerous small oil droplets.

Structure and Composition of the Cell Wall

The relation between cell size and thickness of the cell wall is shown in table 2. The values were obtained by puncturing cells and rinsing them in sea water and then observing a folded region under the microscope. Variations in thickness of the cell wall in different portions of the coenocyte are much more marked in cells taken from the reef than in those kept in the laboratory, where they are protected from abrasion and wave action. Portions of cells having a smaller radius of curvature tend to have a thinner cell wall.

Table 2. *Relation of cell size to thickness of cell wall*
(By direct observation)

Diameter of cell (mm.)	Thickness of wall (μ)	Notes
....	0.9–1.2	17-hour aplanospores; grown in laboratory
0.8	3.3	30 days old; grown in laboratory
1.1	3.5	30 days old; grown in laboratory
2.0	4.0	30 days old; grown in laboratory
3.0	6.2	
6.0	7.4±0.3	
6.2	7.3	
8.0	8.5	
12.0	10.5±0.3	
12.5	13.3	
17×20	12.3	
22.0	15.0±2.0	
30×40	20.0±1.5	
32.0	20.0±1.0	
10×27	6.0 small end 12.0 largest curvature	Irregular (cell 111)

An attempt was made to determine the composition of the cell wall. By means of flotation experiments the density of the wall was found to be 1.55. This value was determined using a solution of magnesium bromide, and is in error owing to the extraction of water from the cell wall.

The weight of the cell wall in relation to its area was ascertained for the cells given in table 3. By comparing values obtained by microscopical measurement with those calculated from a consideration of the dry weight, specific gravity, and area of the cell walls of the same specimens, it was found that about half the volume of the cell wall is lost upon drying. Thus the values given in the last column of table 3 indicate the thickness the cell wall would have if it were solid cellulose.

In preparing the cell walls for weighing, the cells were punctured and rolled between the fingers and rinsed free of cytoplasm with sea water. They were then hurriedly rinsed with distilled water and acetone, blotted with filter paper, and dried at 103° C. to constant weight. The most careful preparations indicate that in this "rinsing process" designed to remove sea salts, a maximum of 1 per cent of the water and acetone extractives are lost.

TABLE 3. *Relation of area to weight of cell wall*

Cell no.	Weight of cell (mg.)	Area (sq. cm.)	Weight of cell wall (mg.)		Vol./area (μ)
			Rinsed	Extracted	
71	17		0.137		
72	139		0.691		
111		10.2	7.70		4.9
67		16.7	19.40	13.4	7.5
93		32.0	36.60	23.0	7.3
92		43.1	58.50	34.0	8.6

If the cell wall after rinsing is placed in slightly acidified distilled water for a few hours, it swells and becomes slimy. Upon further soaking in water the various layers of the wall separate, and with bisected cells it has been possible to obtain 16 hemispheres from the original one.

If the fibrous wall, after soaking in water, is pressed dry with filter paper and again rinsed in acetone, it is found to have lost about ⅓ of the original dry weight of the cell wall (cf. table 3, weight rinsed, weight extracted).

Since the cell wall does not swell or soften in sterile sea water or distilled water containing $Ca(OH)_2$ and does both in distilled water, it seems reasonable to assume that the water-extractable mucilage is calcium pectate.

The values given above were obtained prior to the receipt of publication of the results of Hess, Wergin, Kiessig, Engel, and Philippoff (1939). It is the intention of the author to reinvestigate and refine the procedure employed on *Valonia*.

The extracted cell wall has a calculated thickness, if solid, of approximately ⅓ of its directly observed thickness. It contains in addition approximately ⅓ of its unextracted dry weight (½ the extracted weight) of pectic substance (soluble in distilled water, insoluble in sea water). Assuming the swelling in sea water to be sufficient to give the observed thickness, we would have a cell wall composed of ⅓ cellulose, ⅙ pectin, and ½ water by volume. The pectin is probably more abundant between the layers of the cell wall than between the cellulose fibers of a given layer of the cell wall.

Observations on the Specific Gravities of Cell Constituents

Steward and Martin (1937) have presented data on the relation of the chloride concentration of the cell sap to the size of the vesicles. We have determined the specific gravity of the sap, the cytoplasm, and the entire vesicle as related to cell size.

The specific gravity of the cell sap taken from five cells (nos. 62 to 66) weighing 2.86 to 3.25 g. was determined with a pycnometer and found to have a value of 1.0233 (30°/4°). The sea water in which these cells were living had a value, taken in the same way, of 1.0218 (30°/4°). The variations in specific gravity of sap as related to cell size may be calculated from the data of Steward and Martin (1937) and Steward (1939).

The density of the cytoplasm was determined by flotation in sucrose somewhat more concentrated than isosmotic sucrose and found to average 1.18. This value was obtained after correction for shrinkage caused by the hypertonicity. The value varies with the starch content (density of starch 1.55) of the plastids (see below) and may be somewhat too high in view of the later experiments (see below).

For entire cells the method employed was to place the cell in a graduated cylinder in a known volume of sea water. A mixture of equal parts of isosmotic sucrose and sea water was added from a burette until the cell remained suspended.

The mixture of 50 per cent isosmotic sucrose solution and 50 per cent sea water is so innocuous to *Valonia* that coenocytes kept for two days in such a solution and then punctured formed normal aplanospores in less than an hour. When removed from this solution to 100 per cent sea water 24 hours after spore formation, the spores showed normal growth during the 12 days they were observed.

The values obtained for the specific gravity (30°/4°) of the entire cells are given in table 4. From this table it is evident that the specific gravity of the entire cell decreases with the increasing size of the cell, as might be expected from a consideration of the specific-gravity values of the cell wall, cytoplasm, and cell sap. The values presented are comparable among themselves, but as absolute values are open to some question owing to the difficulties of maintaining standard sugar solutions at Tortugas.

Variation in specific gravity of the entire cell as affected by light is sufficiently great to be readily measurable. Thus, cell 68 in light of 70 foot-candles for 5 hours showed an increase in specific gravity of 0.000029, and cell 69 in the dark for 5 hours showed a decrease of 0.000061, which is a total change in specific gravity of 0.000090 from the original values of the two cells.

The total reducing substances (sugar plus unknown reductants) present in the cytoplasm of these two cells was determined, and after correcting for differences in cytoplasmic volume was found to be 1.65 ± 0.02 arbitrary units for the illuminated cell and 1.05 ± 0.03 units for the darkened cell.

It is evident, then, that in experiments lasting a few hours and involving a knowledge of free sugar content of cytoplasm and/or the specific gravity of the cell, the light conditions must be controlled throughout the experiment.

TABLE 4. *Relation of cell weight to specific gravity of cell*

Weight of cells (g.)	Specific gravity (30°/4°)	Notes
....	1.0993	5¼-hour spores
0.0005–0.001	1.0328	Rhizoids present
0.0005–0.001	1.035	
0.010	1.0336	
0.085	1.0309	
0.545	1.0292	
0.646	1.0290	
1.025	1.0290	
1.500	1.0289	
1.700	1.0286	
5.400	1.0285	

EXPERIMENTS WITH APLANOSPORES

The aplanospores formed by a single *Valonia* coenocyte afford material of fairly uniform constitution, if the spores formed are large compared with the size of the plastids and nuclei contained in the spore. In order to obtain aplanospores of uniformly large size, the relation of the effects of the size of the puncture and the amount of mechanical disturbance of the cell wall to the size and uniformity of the aplanospores was investigated. A small puncture or incision results in the formation of aplanospores of variable size, and generally the process of spore formation is completed quite slowly, although some of the spores may form rapidly. The most satisfactory method for producing fairly large (0.6 to 1.0 mm.) aplanospores of almost identical age (after assuming spherical shape) and of very uniform size was the following: The cell was punctured with one blade of a sharply pointed pair of scissors, the blade being inserted about 2 cm. The wall was then cleanly cut through and the cut extended until the cell was in two halves. The sea water in which the cutting was done was then replaced by fresh sea water.

Kopac (1937) notes that the aplanospores lack a cell wall up to 2.5 hours after spore formation, and that after 3.5 hours a visible wall is present. It is of some interest to know the time of origin of the central vacuole in such aplanospores. If the aplanospores can be centrifuged, the various components may be stratified and their volumes estimated.

If newly formed aplanospores are centrifuged in isosmotic sugar solution, they sink to the bottom; if they are upwards of 2 hours old, they float suspended in mixtures of sea water and sugar solution. By preparing mixtures of sea water and sucrose of graded densities it is possible to investigate the ability of the aplanospores to withstand centrifuging. Cells up to 3 hours after formation invariably burst when centrifuged at 2000 r.p.m. and 12 cm. radius for 10 minutes. Cells 3.5 hours old or older have a wall sufficiently strong to prevent bursting at these forces. If one excepts the relatively weak plasma membrane, there was no evidence that an elastic cell wall capable of being stretched markedly was present in any of the stages up to or over 2 hours old.

In aplanospores 5.5 hours old it was found that centrifuging resulted in stratification of the plastids at a level equal to 70 per cent of the entire aplanospore volume. The hyaline cytoplasm and nuclei are distributed in a layer centripetal to this, representing an additional 7 per cent of the aplanospore volume. Assuming that the plastids are spheres of uniform diameter and noncompressible, there would be an additional 26 per cent of the space between plastids filled with hyaline cytoplasm. Thus the hyaline cytoplasm would represent $70 \times 0.26 + 7$ per cent $=$ 25 per cent of the aplanospore volume, and the plastids 52 per cent of the volume, leaving 23 per cent for the vacuole. The ratio of plastids to cytoplasm estimated in this manner gives 68 per cent of the total protoplasmic volume (less vacuole) as plastids, which compares with 60 to 65 per cent given by Doyle (1935) for mature cells.

If one considers the specific gravity of similar aplanospores (5.25 hours old) from the same cell, one finds that the parent cytoplasm of specific gravity 1.18 has given rise to an aplanospore of specific gravity 1.099. Assuming a sap of specific gravity 1.023 (which is lower than might be expected), it would require a vacuolar volume of over 50 per cent to bring the cytoplasm containing it (neglecting the cell wall) to a specific gravity of 1.099. Direct observation shows that the vacuole cannot have these proportions. Granting even that the value 1.18 for the specific gravity of the cytoplasm at the start of the experiment is somewhat high, it must be markedly reduced during the formation of the spore.

General Observations on the Metabolism of Valonia

If cells are kept in diffuse light their plastids contain visible starch of the appearance shown in figure 1, *B*. Similar cells kept in the dark for 14 days show a marked reduction in starch content and an increase in oil droplets, as shown in figure 1, *C*. For a discussion of the origin of these oil droplets see Doyle (1935).

The variation in amount of free reducing substance in light and in darkness has already been mentioned. Some indication of the rate of starch metabolism in *Valonia* is given by the following experiment: If the cytoplasm of a cell is concentrated by centrifuging, it can be transferred to distilled water, whereupon the plastids swell and burst, liberating their contained starch grains. Upon centrifuging at high speeds (air turbine), the starch collects in the bottom of the centrifuge tube and can be estimated volumetrically. Two spherical cells of approximately 1 ml. volume had been kept under identical conditions in the laboratory for three weeks. One of these was left in its usual place, the other was removed to a dark box for four days. At the end of this time both cells were cut open and the starch estimated as described above. The cell which had been in the light had 5 ± 1 mg. of starch, and the one in the dark had 1 ± 0.3 mg. of starch at the end of the experiment. This indicates a utilization of roughly 1 mg. of starch per day over a four-day period. Other experiments indicate that the utilization of starch on the last day is much lower than on the first day in darkness. Not all the starch may be considered as burned, since fat is known to increase under these conditions. The transformation to fat supplies some of the needed oxygen. This would explain in part why Steward and Martin find that the oxygen uptake in the dark is so low. Furthermore, if one takes into account the volumes of actively respiring cytoplasm in *Valonia* and in the forms noted by Steward and Martin (viz., by deducting vacuolar volumes), the order of magnitude of *Valonia* metabolism is not so strikingly different from that of the barley-root and potato-slice tissues as would appear from the values given for oxygen uptake. On the other hand, experiments on the peptidase and amylase activity of the cytoplasm indicate a low concentration of enzymes, which is compatible with low metabolic activity.

Summary and Conclusions

Measurements have been made of the relation of the volume and thickness of the cytoplasm and cell wall to the size of the coenocyte.

The specific gravities of various cell constituents and of cells of various sizes have been measured.

From a consideration of the results presented it would appear that the level of metabolic rate in *Valonia* is of a low order, but not necessarily of a different order of magnitude from that of the barley-root and potato-slice systems.

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PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

X

Regional Photosensitivity and Photoreceptors of Crangon armillatus and the Spiny Lobster, Panulirus argus

By Walter N. Hess

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With one plate and two text figures

[ISSUED SEPTEMBER 14, 1940]

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Regional Photosensitivity and Photoreceptors of Crangon armillatus and the Spiny Lobster, Panulirus argus

Introduction

As shown by Prosser (1934) and Welsh (1934) working independently, crayfish from which the eyes have been removed are sensitive to light in the region of the sixth abdominal segment, but no responses occurred when other regions were illuminated. Although no photoreceptor cells were found by either author, Prosser concluded on the basis of regional photosensitivity that the sixth abdominal ganglion was the seat of photosensitivity and hence was the "caudal photoreceptor." Because of the backward swimming habits of this animal, the apparent importance of this caudal photoreceptor in influencing the behavior of the animal was at once recognized.

The discovery that freshly molted *Crangon armillatus* and spiny lobsters (*Panulirus argus*) are sensitive to light in other regions of their bodies, in addition to the sixth abdominal segment, led to this investigation.

Materials and Methods

The eyes were removed from all animals before study except those of the larvae of *Crangon*. In order to prevent bleeding, the eye stalks of the spiny lobsters were ligatured near their bases before the distal portions were removed. Because of the shortness of the eye stalks of *Crangon* the eyes were removed by means of fine scissors without the use of ligatures. Young and old individuals of both species were used. Some of these animals had just molted while others had not molted for a considerable period. Consequently, some had soft exoskeletons containing comparatively little pigment while others, especially the spiny lobsters, had hard, densely pigmented exoskeletons. Since *Crangon armillatus* molts, on an average, about every twelve days, its exoskeleton is always soft and comparatively free from pigment. All animals were dark-adapted before being used in these experiments by being placed for one hour or longer in a dark room.

Some of these animals from which the eyes had been removed were placed in small aquaria having black backgrounds and studied in the free-swimming condition. Others were supported in an upright position against black paraffined plates and held firmly by rubber bands in such a position that the movements of the respiratory appendages and walking legs were undisturbed. The water in the aquaria was kept at a temperature of approximately 29° C. A small beam of light of rather high intensity was used as the source of illumination for determining the location of the photosensitive regions of these animals (Hess, 1924). By using different lenses and screens, beams of light of different sizes and intensities were obtained.

Regional Photosensitivity of Crangon armillatus

Crangon armillatus is nocturnal in habits and lives in burrows in the sand of the Florida coastal waters. By using the light referred to above it was found that freshly molted animals are very sensitive to light. These animals respond by moving one or more of their walking legs when the dorsal and lateral surfaces of their bodies including the abdomen, cephalothorax, uropods, telson, and to a less extent the legs, antennae, and antennules are exposed to light. Although the abdomen appears to be slightly more photosensitive than the cephalothorax, no appreciable difference was observed between the photosensitivity of the sixth abdominal segment and that of the other segments of the abdomen.

As shown in plate 1, figure 1, the exoskeleton of *Crangon* contains very little pigment. Even twelve days after molting *Crangon* is usually still photosensitive in many regions of its body. No part of its body, however, is as sensitive to light at this time as just after molting. Photosensitivity in these animals appears to be directly associated with the fact that they develop comparatively little pigment in their exoskeletons.

Irrespective of the length of time after the last molting period, no responses were obtained when the outer margins of the uropods were illuminated. Responses were obtained, however, when the proximal two-thirds of these appendages were exposed to light.

Old animals that have not molted for two or three weeks react to illumination much less readily than do freshly molted animals. If they are illuminated in the more pigmented regions they may or may not respond, but if the less pigmented areas of the dorsal and lateral regions of the body are illuminated the animals usually react. In general, the more pigment there is in the exoskeleton, the slower and less definite are the responses.

For several days following hatching the larvae of *Crangon* live at the surface of the ocean. When these larvae having normal eyes are dark-adapted and then exposed to light varying in intensity from 0.0012 to 500,000 foot-candles, they orient immediately and swim toward the light with their caudal ends foremost. This behavior suggests the possibility of a caudal photoreceptor. However, if one or as many as five of the abdominal segments are removed they still orient and swim toward the light with the caudal end foremost. Specimens in which the eyes were destroyed by dissecting needles were no longer able to orient to light, but they did show evidence of being sensitive to light even though the caudal segment of the abdomen had been removed.

Regional Photosensitivity of the Spiny Lobster

Unlike *Crangon,* the spiny lobster (*Panulirus argus*) develops a heavy, pigmented exoskeleton. However, it remains soft and contains a comparatively small amount of pigment for two or three days following molting. During this period many regions of the body are sensitive to light (Hess, 1938). The regional photosensitivity of a spiny lobster that has recently molted is similar to that of an adult *Crangon* that has not molted for about two weeks. However, instead of all the abdominal segments being about equally photosensitive, the sixth segment of the spiny lobster is more photosensitive than the others. The older these animals become, the more pigmented their exoskeletons become, and consequently photosensitivity is gradually reduced in such animals. Even immediately after molting photosensitivity is often not very pronounced. Very old individuals with densely pigmented exoskeletons usually do not respond by moving one or more of their walking legs when light, of the intensity used in these experiments, is directed upon them from above. If their abdomens are flexed, however, so that the intersegmental regions are exposed, these animals often respond to a horizontal beam of light from the rear. This is especially true when the abdomen is partially flexed and the intersegmental region between the telson and the sixth abdominal segment is illuminated.

Young spiny lobsters are sensitive to light in many regions of their bodies, especially if they have recently molted. These young animals have areas in their exoskeleton containing very little pigment, which are bordered by rather densely pigmented areas, as is shown in plate 1, figure 2. As one might expect, the more pigmented areas are less photosensitive than the areas having a smaller amount of pigment.

Freshly molted *Crangon* and spiny lobsters avoid strong lights and remain in seclusion for some time following molting. Attempts to test the ability of freshly molted animals from which the eyes have been removed to orient to light show that they usually avoid the light by random movements. However, if illuminated by a horizontal beam of light from the rear they usually move directly away from the light. When the abdomen is illuminated from the side the animal often turns its abdomen from the light. Likewise, when the cephalothorax is exposed to lateral illumination the anterior part of the body often turns away from the light. Yet there is no regularity in the nature of these responses.

Effect of Certain Operations on Responses to Light

In order to ascertain more definitely whether the sixth abdominal ganglion is the only photosensitive structure in these animals from which the eyes have been removed, certain operations were performed on freshly molted adult *Crangon* and spiny lobsters.

If a lengthwise incision is made on one side of the median ventral region of the fifth and sixth abdominal segments and a piece of black cardboard is inserted above the sixth abdominal ganglion, these animals will still react if the abdomen, as well as certain other regions of their bodies, is exposed to light. If the ventral nerve cord is cut between the fifth and sixth abdominal ganglia and the animals are then exposed to light, they respond much as before the operation except that they are unable to move the telson and uropods. This shows that photosensitivity in these animals is not limited to the sixth abdominal ganglion.

Comparison with Other Species

In connection with this investigation certain observations were also made on the American lobster (*Homarus americanus*) and the common crayfish (*Cambarus affinis*).

American lobsters with hard exoskeletons, from which the eyes have been removed, did not react by moving any of their walking legs when illuminated from above. Unlike many of the spiny lobsters that were studied, they gave no response when the sixth abdominal segment was illuminated, which may be due to the fact that the entire dorsal surface of this segment is heavily pigmented. Freshly molted American lobsters, however, are sensitive to light in many regions of their bodies. This photosensitivity is not as pronounced as in the case of *Crangon* or the spiny lobster, since these animals, even immediately after molting, have a large amount of pigment in their exoskeletons.

Crayfishes with hard exoskeletons, from which the eyes have been removed, react to light only when the sixth abdominal segment is illuminated, as shown by Prosser (1934). Freshly molted individuals, however, react when other regions of their bodies are exposed to light.

Photoreceptors

In order to determine the nature of the organs in these animals that are stimulated by light, a special study was made of certain regions of the body of *Crangon armillatus*. Because of a difference in the regional photosensitivity of the uropods of this animal, these organs were selected for special study. Vital methylene blue preparations (Hess, 1937) were made of the uropods, which show many nerves with nerve-cell bodies and varicosities (fig. 1). As will be seen by consulting this figure, the nerves that enter the uropods extend to the periphery, where they enter spines or filaments. By means of the small beam of light it was found that the uropods are not photosensitive along their margins, but they are photosensitive in the region of the cell bodies of these neurons. Be-

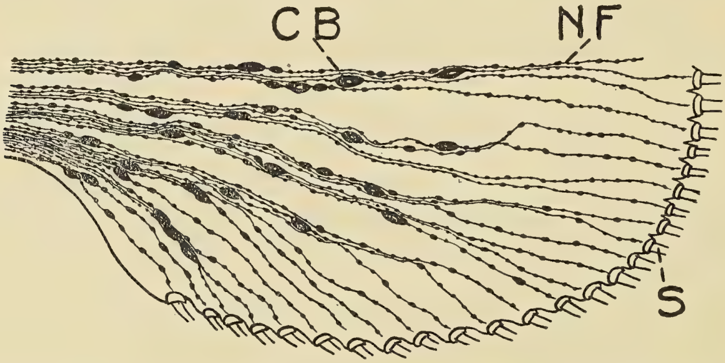

Fig. 1. Drawing of a portion of uropod of *Crangon armillatus* following vital methylene blue staining. *NF*, nerve fiber with varicosities; *CB*, cell body of neuron; *S*, spine.

cause of this difference in photosensitivity and because these tactile neurons are the only sensory structures that stain with vital methylene blue in this region, it seems very probable that the cell bodies of the neurons that extend to the peripheral spines are the photosensitive elements.

A careful study of the neuro-sensory structures in the dorsal body wall of segments five and six, after staining with vital methylene blue, does not reveal any differences in the sensory elements of these two segments. Sensory elements of the type illustrated in figure 2 were found near the dorsal body epithelium in both segments, but no other types of sensory element were found here. Since some of these were found to be connected with spines, it is probable that they are chiefly tactile in function. However, it is also probable that these sensory elements are also sensitive to light and that they function as the photoreceptors of these regions.

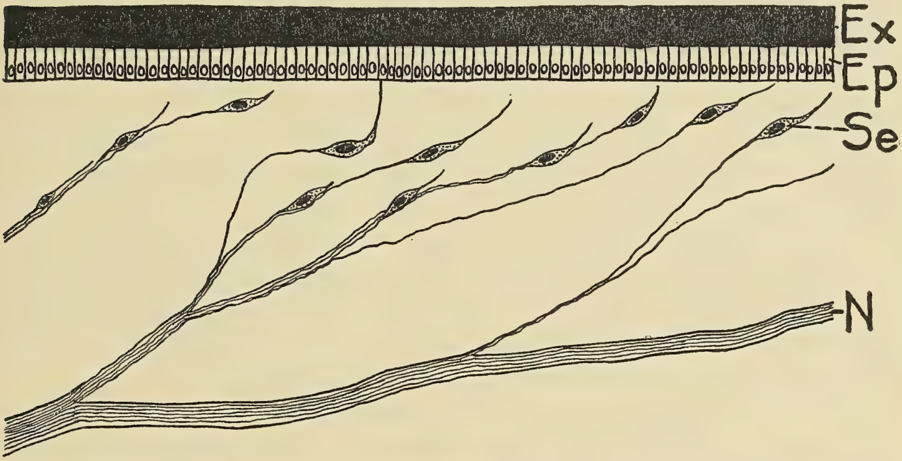

FIG. 2. Drawing of sensory elements in the dorsal body wall of *Crangon armillatus* from sections of the sixth abdominal segment that had been stained with vital methylene blue. *Ex,* exoskeleton; *Ep,* epithelium of body wall; *N,* nerve; *Se,* sensory element.

It is possible that specialized photoreceptor cells may still be found in these animals. However, this work strongly suggests, especially in the case of the uropods, that the photoreceptors are the cell bodies of neurons which may also serve a tactile function, and that they are more photosensitive when there is little or no pigment in the exoskeleton.

As stated previously, Prosser (1934) concluded on the basis of localized photosensitivity in the crayfish that the sixth abdominal ganglion is the seat of photosensitivity after the eyes have been removed. Although a study was made, by means of special neurological technique methods, of the nerve-cell bodies in the sixth abdominal ganglion, none were found that appeared to be morphologically modified as photoreceptors. It seems very probable that we have here a condition similar to that of the uropods described above, in which certain nerve-cell bodies are stimulated by light without being morphologically modified as are the photoreceptors of earthworms and certain other animals. That nervous tissue is stimulated by light is a well-known fact. For example, illumination of the optic nerve results in endocrine responses (Benoit, 1935).

Summary

1. *Crangon armillatus* is usually sensitive to light in many regions of its body, irrespective of how much time has elapsed since the last molting period.

2. Freshly molted spiny lobsters (*Panulirus argus*) are sensitive to light in many regions of their bodies.

3. Old spiny lobsters, with hard exoskeletons, from which the eyes have been removed are usually not sensitive to light of the intensity used in these experiments.

4. The margins of the uropods of freshly molted *Crangon armillatus* and spiny lobsters are not sensitive to light, but the basal two-thirds of these appendages are sensitive to light.

5. Adult *Crangon armillatus* and recently molted spiny lobsters react when illuminated from above after the sixth abdominal ganglion has been shielded by black cardboard and also after the ventral nerve cord has been cut between the fifth and sixth abdominal segments. This shows that photosensitivity in these eyeless animals is not limited to the sixth abdominal ganglion.

6. Newly hatched *Crangon armillatus* with normal eyes swim toward the light with their caudal ends foremost irrespective of the number of abdominal segments that have been removed.

7. *Crangon armillatus* and spiny lobsters from which the eyes have been removed do not usually orient to light, but respond by random movements. When their bodies are heavily pigmented, or if they are in poor physical condition, they usually do not respond at all. However, if they do respond their responses are usually much slower than those of freshly molted animals in good physical condition.

8. The sixth abdominal segment of these eyeless spiny lobsters and crayfishes is the most photosensitive region of their bodies. However, in *Crangon armillatus* and the American lobster (*Homarus americanus*) all the abdominal segments appear to be equally sensitive to light.

9. On the basis of regional photosensitivity of the uropods, it seems probable that the cell bodies of the neurons which connect with the peripheral spines are sensitive to light and hence function as photoreceptors.

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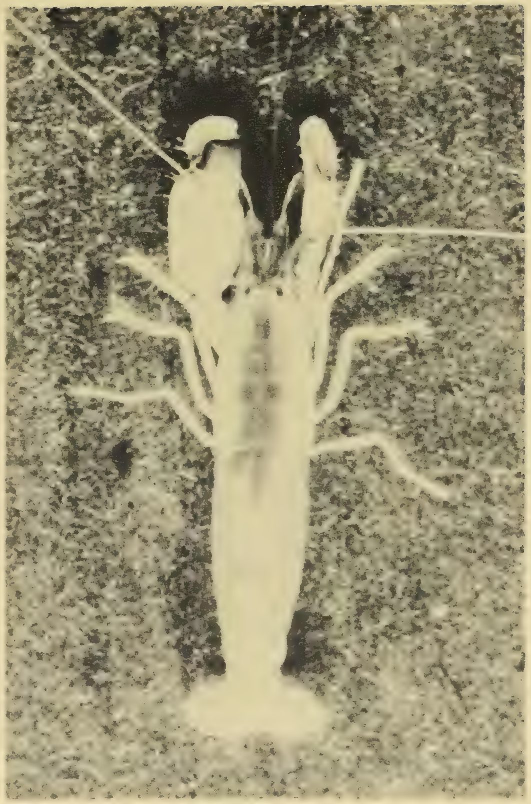

FIG. 1. Dorsal view of a mature *Crangon armillatus* 10 days after molting. Observe lack of pigmentation in exoskeleton.

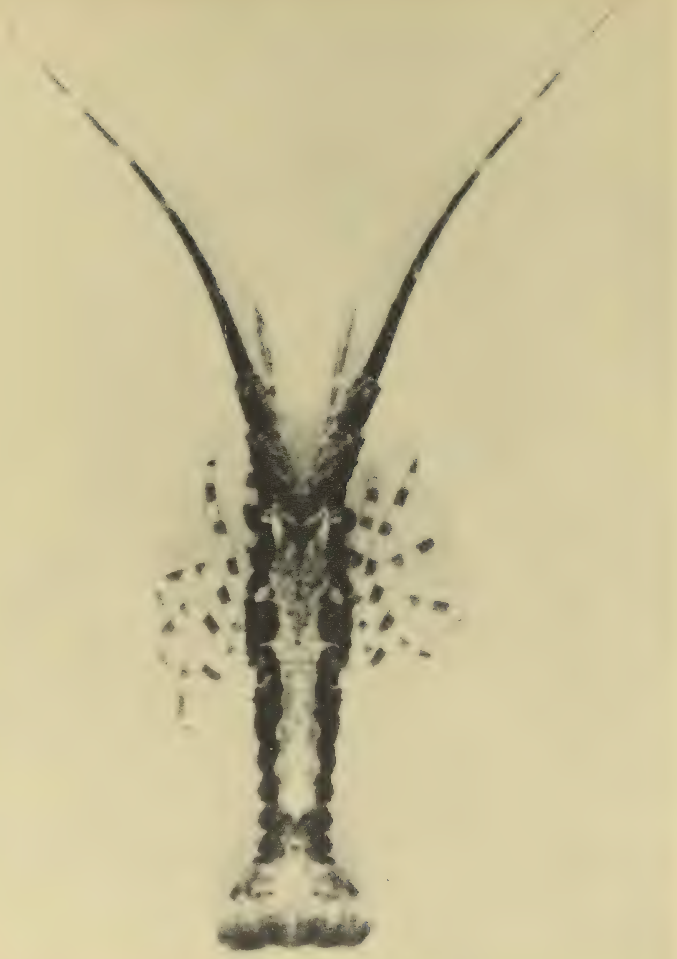

FIG. 2. Dorsal view of young spiny lobster. Observe distribution of light and darkly pigmented areas of the exoskeleton.

XI

Native Cellulose in the Ascidian Phallusia nigra

By John Spence and Oscar W. Richards

Research Laboratories, Eastman Kodak Company, Rochester, New York
and Research Department, Spencer Lens Company, Buffalo, New York

With one plate

[ISSUED SEPTEMBER 14, 1940]

Native Cellulose in the Ascidian Phallusia nigra

Many organic compounds of high molecular weight are readily identifiable from the characteristics of their X-ray diffraction diagrams. Essentially this method consists in computation of the interplanar spacings, appraisal of the intensities, and subsequent comparison with recorded values. Such a method is reasonably rapid and reliable, and can be performed with little or no structural alteration of the specimen.

Cellulose and its derivatives have been extensively examined by X-ray diffraction methods in the search for a complete solution of the structure and crystallite arrangement of the cellulose molecule. Of special interest is the classic study of *Valonia ventricosa* by Sponsler (1930) and the examination of native cellulose of *Phallusia mamillata* and *Mogula crystallina* by Herzog and Gonell (1924). From the latter, the presence of native cellulose in the animal kingdom was again established. Mark and Susich (1929) also examined this source of native cellulose, and directed attention to the valuable information derivable from materials which showed a preferred crystallite orientation and were deformable under stress. Recently the value of another ascidian, *Ciona* sp., has been demonstrated by Gross and Clark (1938) in an interpretation of the refinements of the cellulose structure.

From the analytical standpoint, X-ray diffraction diagrams not only confirm the initial chemical identification of cellulose by Schmidt (1845), but they also show the presence of crystallites and their orientation.

The *Phallusia nigra* was collected in the moat of Fort Jefferson and the tunic was removed on return to the Tortugas Laboratory. In the meantime the animals were kept in sea water. Longitudinal and circular strips of about a centimeter in width were cut from the tunic in regions which were free from incrustations and placed in FAA (100 ml. 50 per cent alcohol, 6.5 ml. formalin, and 2.5 ml. glacial acetic acid). The strips were washed and dried both with and without tension.

Table 1 gives the measurements of the spacings of the diagrams as computed from the Bragg relation

$$n\lambda = 2d \sin \theta$$

in which λ is the wave length of the radiation employed, θ is the glancing angle, and d is the spacing value in angströms. Three different diagrams designated numerically are characterized. No. 1 was given by the specimen dried without tension; in no. 2 the specimen was dried under tension with the X-ray beam normal to the plane of the test; and no. 3 was treated similarly to no. 2 except that the X-ray beam was in the principal plane of the sheet. Comparison with the recorded figures and intensities employed by Andress (1929) at once identifies the presence of native cellulose in the test.

Diagrams 2 and 3 showed marked fibering of the 101 reflection, as did another

TABLE 1

Native cellulose (Andress data)				Phallusia nigra						
				No. 1, dried without tension		Dried under tension				Average of nos. 1, 2, 3 d°_{A}
						No. 2		No. 3		
Interference	Index	Intensity	d°_{A}	Intensity	d°_{A}	Intensity	d°_{A}	Intensity	d°_{A}	
A_1	101	Strong	5.94	Average	6.06	Strong	6.08	Strong	5.97	6.04
A_2	$10\bar{1}$	Fairly strong	5.36	Average	5.39	Fairly strong	5.31	Fairly strong	5.34	5.35
II_1	021	Average	4.30	Average	4.28	Average	4.27	Average	4.29	4.28
A_4	002	Very strong	3.89	Strong	3.90	Very strong	3.84	Very strong	3.86	3.87
II_3	$\left\{\begin{matrix}221\\122\end{matrix}\right\}$	Weak	3.07	....	....	Weak	3.14	Weak	3.08	3.11
II_4	$\left\{\begin{matrix}22\bar{1}\\122\end{matrix}\right\}$	Weak	2.88	Very weak	2.91	Weak	2.81	Weak	2.82	2.85
I_2	$11\bar{1}$	Weak	2.58	....	2.65	Weak	2.51	Weak	2.58	2.58

diagram of the unstressed samples with the X-ray beam in the plane of the sheet. Similar manifestations have been given by the other ascidians examined by this method.

The result, namely, the recognition of native cellulose and the preferred orientation of the crystallites in *Phallusia nigra,* is naturally anticipated from previous observations on other ascidian tests. This method provides a useful analytical "tool" for use in zoological investigations.

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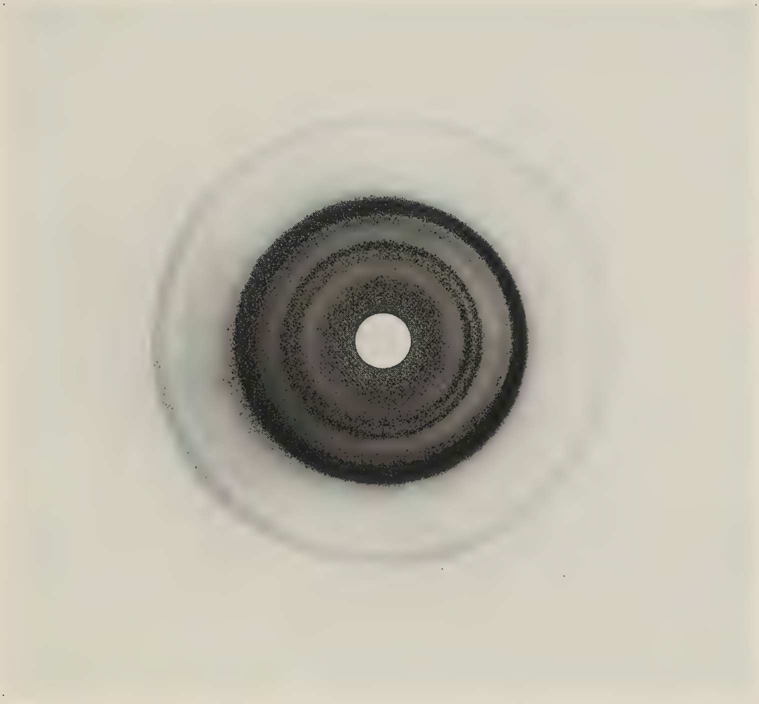

X-ray diffraction diagram of *Phallusia nigra*

PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

XII

Chromatophore Reactions of Embryos and Larvae of Pomacentrus leucostictus

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Brooklyn College

With four plates

[ISSUED SEPTEMBER 14, 1940]

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Chromatophore Reactions of Embryos and Larvae of Pomacentrus leucostictus[1]

INTRODUCTION

Interest in the origin of the changing color patterns of fishes and certain other vertebrates, together with certain questions that have been raised by the work of other investigators in their study of this problem in embryos of vertebrates, prompted an investigation of the color mechanism in developing fishes. Both embryos and larvae of *Pomacentrus leucostictus,* which is found in abundance in the region of the Dry Tortugas, were used in this study. For making possible this investigation at the Tortugas Laboratory, the writer gives grateful acknowledgment to the Carnegie Institution of Washington.

According to the reports of Bancroft (1912), Spaeth (1913), and Gilson (1926), *Fundulus* young at the time of hatching have the ability to change color. It does not necessarily follow, however, that this is true of the young of other fishes. Becher (1929) observed the young of *Coregonus* and two species of *Salmo* in an attempt to find color responses in fishes at stages earlier in development than that reported for *Fundulus.* He found that the embryos of these three fishes often show color changes, and concluded that the ability to change color is manifested soon after the pigment makes its appearance in the developing embryo. A similar view was reached by Duspiva (1931) in a study of color change in the young of *Salmo salvelinus* and *Perca fluviatilis,* and also by Tomita (1936), who studied the embryos and larvae of *Macropodus opercularis.*

The work of Babák (1910) on the Mexican axolotl larvae makes possible the dividing of the color change reaction into two phases. One is the primary phase, during which the animal is pale when in total darkness and dark in tint when in light. Through the secondary phase, the animal has functional eyes and is dark in tint when in total darkness; it is either pale or dark when in light, depending upon its surroundings. It is believed that while the animal is in the primary phase its color bodies respond directly to the stimulus, and that in the secondary phase they respond indirectly to the stimulus received through the eyes. According to Tomita (1936), the embryos and young larvae of *Macropodus* demonstrate the primary phase, and the secondary phase begins several days after hatching and persists on through maturity. Thus far, except for the report of Parker (1936), who states that the newly born young of *Mustelus* omits the primary phase and is born with a melanophore system quite like that of the adult, the works of other investigators are in general agreement that embryos and larvae of certain fish and amphibians have the ability to change color and to exhibit first the primary and then the secondary phase. There is need, however, for further study of this problem, especially in the young of other fishes, before the pigmentary picture is by any means complete. Assuming that, with the exception of axolotl, the adults of the young referred to in the reports cited possess the ability to change color, the writer finds that the adult *Pomacentrus* differs from the other adults referred to in that it does not change color. This fish lives over a white background of coral which in places takes on a brown or dark hue due to decaying vegetation and to other materials such as dark shells and occasional dark pieces of the coral; yet its color over the entire body, except for the fringe of yellow on its fins, is a dark blue. The writer has never observed any variation of this color in these adults, even over various backgrounds in the field.

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[1] In part with the support of a grant from the American Philosophical Society.

Development of Pigment

During the summer months a great many eggs are deposited by *Pomacentrus* females on a meshwork of fibrous material inside of conch shells. The egg-laying requires one or two days or longer and, since the young will hatch in about four days from the developmental stage represented in figure 1, it is possible to find in a single conch shell all stages from the very beginning to hatching. In some instances conch shells containing several hundred *Pomacentrus* embryos were taken into the laboratory, and at other times the embryos in the desired stage of development were scraped off the conch shells in the field and then taken into the laboratory. The female fish discharges each egg within a capsule, as is shown in figures 2 to 4 inclusive. Attachment to the fibrous substratum of the conch shell is made by cementation of the brushlike fibers on one end of each capsule (figs. 22, 24). During the development the head of the embryo is first at the basal end of the capsule (figs. 2–4); but as development continues and at the time when the eyes are fully formed the embryo turns itself, and remains until it hatches with its head at the tip of the capsule (figs. 13, 22, 24).

Several of these capsules containing embryos were separated one from another and were kept in finger bowls for observation in the laboratory. The first appearance of pigment occurs at a stage in development when the eyes are beginning to form. This pigment can be recognized as a large melanophore over each eye region and also on each side of the trunk of the embryo near its heart (figs. 2–4). Within a few minutes after these large melanophores can be seen, smaller ones appear over the yolk sac (fig. 4). In a short time other melanophores are formed, numerously over the head and sparsely at first over the body region of the embryo and on the yolk sac (fig. 5). Later these pigment bodies are conspicuous along the lateral line region (figs. 8, 9), and form a definite line along the ventral region of the tail (fig. 15). Within two days after the stage shown in figure 1, both the dorsal and ventral median fins are formed. At this time yellow chromatophores develop on the anterior part of the dorsal fin, while brown, anastomosing chromatophores appear on the posterior part of the dorsal fin and all over the ventral fin (figs. 7–9). Within a day before hatching, some of the melanophores on the trunk region of the embryo migrate out onto the dorsal and ventral fins (fig. 8). When the embryo hatches and breaks free of the capsule, the head region is covered with melanophores, the trunk has melanophores along the lateral line and at the bases of the two median fins, the tail has a line of melanophores along its ventral region, the median fins are covered by melanophores as well as by yellow and brown pigment bodies, and the paired ventral fins, which have made their appearance, are covered with melanophores and brown chromatophores (fig. 6). The melanophore behavior pattern of these young embryos is one of non-responsiveness up to hatching time. The majority of the melanophores are in the stellate stage during this prehatching period (figs. 28, 29).

Methods and Experiments

Several embryos within their capsules, which had been pulled free of the fibrous materials on the conch shell, were kept in finger bowls immersed in running sea water, or in finger bowls on tables. The sea water in the latter bowls was changed twice each day. Embryos from a single conch shell, presumably from a single female, were placed in several finger bowls to constitute a single experiment. In each experiment embryos were kept over a black background, over a white background, and in total darkness, and were also observed during pigment development while on light-colored tables. To insure uniform illumination each day, a 50-watt lamp was kept from 7:00 A.M. to 10:00 P.M. above the embryos which were over the black and the white backgrounds. Care was taken to eliminate as much as possible the effects of mechanical stimulation during the handling of the embryos while they were being photographed. All photographs were taken very quickly and within a few seconds after the embryos had been transferred from the finger bowls to watch glasses. Many observations on the degree of contraction or expansion of individual melanophores on a single embryo, as well as observations of the degree of paleness or darkness brought about by the melanophore action on small groups of embryos, were made and recorded as photographs. Active larvae were observed first through a microscope and then narcotized for a few seconds while being photographed. As far as it was possible to estimate the condition of the melanophores before and after narcotization, this treatment had no effects upon these pigment bodies.

Reactions of Embryos over White Background

Some embryos were kept in finger bowls which had been painted previously on the outer surface with a white flat paint. The observations began when the embryos first developed pigment and were continued at frequent intervals until three or four days after they had hatched. Most of these embryos were allowed to develop freely without any physical modification, while others were treated so as to prevent eye development. The eyeless ones were obtained by applying pressure on the capsules at the head region of the developing young when they were at a developmental stage between those shown in figures 1 and 2. Eye formation was prevented by this method in a small percentage of the attempts. The eyeless embryos developed in the usual manner except for their lack of eyes, and hatched along with the normal ones. During a period of time from the beginning of development up to a few hours before hatching there was considerable variation between the melanophores of one specimen and those of another, whether normal or eyeless specimens. Up to the time of hatching, a few embryos had expanded melanophores, while a few others showed very definitely contracted pigment bodies. The melanophores of the majority of the embryos kept over a white background, however, were in the stellate form during this period of development (fig. 27). A conspicuous change in the color picture of the embryos over the white background took place just prior to hatching and lasted on through the hatching period, to change again some few hours after the larvae were free-swimming. Most melanophores in all specimens passed into the contracted condition just a few hours before the young hatched. This was equally true of the normal embryos (fig. 11) and of those without eyes (fig. 10). It can be seen from these illustrations that the melanophores are maximally contracted at this time, causing a paling of the specimens. Within a day after hatching the melanophores failed to show uniformity in their response. Some did contract, as shown in figure 19; but these were not uniformly contracted over the body of a single larva. Even this amount of contraction was found in comparatively few of the larvae. Lack of uniformity in the degree of pigment concentration or dispersion characterizes the melanophore picture of these older larvae.

In observations of the movement of pigment within the other chromatophores, absolutely no consistency could be found in the response to a white background. In some embryos and larvae the yellow chromatophores were contracted while the brown ones were expanded (fig. 7). In other specimens both the yellow and the brown pigment bodies were contracted, while in still others both were expanded.

Reactions of Embryos over Black Background

Specimens were kept in finger bowls which had been painted on the outer surface with black paint. These bowls, containing specimens from the same batches as those kept in the white bowls, were placed alongside the white bowls and under the same illumination. In these specimens the melanophore picture up to a few hours before hatching was similar to that of those which were kept in the white bowls. During this early development, both in the normal and in the eyeless specimens, the melanophores were for the most part in the stellate stage. Some contracted ones could be found, however, as well as some fully expanded ones (fig. 23). Within a few hours before hatching the melanophores of both the normal and the eyeless embryos became expanded, the expansion being slightly greater, on the whole, in the normal embryos than in the eyeless ones (figs. 12, 13). There was still evidence of melanophore response to a black background after the normal larvae (figs. 18, 21), as well as the eyeless ones (fig. 20), hatched. Later in development, at a time corresponding to that of the second change in the larvae over the white background, the melanophores of the larvae over the black background failed to respond to their environment. A lack of uniformity was again observed. Contracted melanophores were found in some of the older larvae, and expanded melanophores in others (fig. 15).

The yellow and the brown chromatophores in the embryos which were kept over the black background gave the same response as they did in those over the white background. In some specimens very definitely contracted yellow bodies and expanded brown bodies could be seen. In other embryos and larvae the yellow chromatophores were expanded while the brown ones were contracted. In some specimens both the yellow and the brown chromatophores were expanded, while in others both were contracted.

Reactions of Embryos in Total Darkness

Embryos, both eyeless and normal, from the same batches of developing young that were used in the white and the black background experiments were placed in clear finger bowls and kept in a dark room during the same time and for the same length of time as in the previously described experiments. The behavior of both the normal and the eyeless young kept in darkness was the same as that reported for those kept over the white and the black backgrounds. Their color picture up to within a few hours before hatching is characterized by lack of uniformity in the degree of melanophore contraction or expansion. In general the melanophores were in the stellate condition (fig. 25). Just as the embryos were beginning to hatch, all their melanophores became almost instantly contracted (fig. 22). The suddenness of this conspicuous reaction is illustrated in figure 24, this photograph having been taken a few minutes earlier than the one shown in figure 22. This contraction phase continued on through the hatching period (fig. 14), to cease, almost as suddenly as it had appeared, a few hours after the larvae were swimming about in the bowls (fig. 17). The melanophores of these specimens were slightly more contracted than were those of the young kept over the white background.

Other Experiments

During the handling of older embryos in removing them from the meshwork of materials on the conch shells, it was noticed that in most cases the melanophores would contract. Also, while pressure was being applied with forceps to the very young embryos in their capsules in order to inhibit the formation of eyes, almost instantaneous contraction of the melanophores was observed. Embryos in all stages of development up to the hatching stage were stimulated mechanically either by applying pressure to the capsule as previously described or by pricking the older ones with needles. All these specimens reacted to the stimulus by contraction of their melanophores (figs. 5, 26). In contrast, the normal melanophore picture of the non-stimulated animal is shown in figure 28. The other chromatophores were not studied in this experiment.

Both young and older embryos were subjected to low temperature by being kept in a cold chamber at about 8° C. for an hour or less. In response to this environment all melanophores contracted. If the embryos were removed from the cold chamber before the lapse of about 30 minutes, the melanophores would, after several hours outside the cold chamber, return to the stellate stage.

A small cut was made across and through the lateral line of several older larvae. Since many melanophores are distributed along this organ and since these pigment bodies did not in every instance react in the same way as did others on the trunk region, it was believed that this nervous organ might have some control over these melanophores. The cutting of the lateral line resulted in the contraction of the melanophores only at the injury. All other melanophores both anterior and posterior to the cut remained as they were previous to the experiment, that is, either in the stellate condition or expanded (fig. 16).

Discussion

At what point in the development of the young of *Pomacentrus* do the melanophores become active? This question has two implications. One is concerned with the exact time when the melanin actively moves within the melanophores in response to direct stimulation, before the embryo has developed eyes; the other deals with the time when the melanophores respond to indirect stimulation through the eyes or some other mechanism. With regard to the first implication, it was found that very soon after the appearance of melanophores, a few hours later than the stage shown in figures 2 to 4—that is, during the early embryo stage—the melanin migrates within the melanophores. It is to be noted here that the embryonic life of this fish, as observed when kept in the laboratory, is only four days in duration. The observation that the melanophores respond to stimulation at a very early stage is borne out by the consistent results obtained when the embryos were subjected to cold or to body pressure. In every instance the melanophores all over the body and yolk sac of the stimulated specimens contracted. It is believed that the individual melanophores respond directly to the stimulation and not through any intervening system or mechanism. As to the second implication of the question, that is, the time of the melanophore response to stimulation received indirectly, the answer proved to be that this occurs in the late embryo stage, just before the embryos hatch. When the embryos reached this stage in development their melanophores acted as a unit in response to certain environments: they contracted when the embryos were over a white background (figs. 10, 11); they expanded when the embryos were over a black background (figs. 12, 13); and they contracted when in a dark room (fig. 22). Precisely the same results were obtained from both normal and eyeless embryos in all three situations. Both the sudden appearance of this reaction and its short duration seem to the writer to be significant.

How does the melanophore picture of the embryos and larvae of *Pomacentrus* fit into the picture given by the reports of other investigators in regard to their work on other fish embryos and larvae? The melanin does move within the melanophores of the embryos of *Pomacentrus* immediately after these pigmentary organs are completely formed. In this respect the present observations are in agreement with those of Duspiva (1931) on *Salmo salvelinus* and *Perca fluviatilis,* and with the conclusions of Tomita (1936) concerning *Macropodus* embryos. The writer could find, however, no direct response of the melanophores to light. In this particular respect his observations differ from those of Tomita. No uniformity was found either in the concentration or in the dispersion of pigment granules within the melanophores of a single embryo, or in the general effect of these granules upon the tint of specimens in a single bowl under illumination, whether over a black or a white background. The melanophores of these young embryos of *Pomacentrus* did respond in a coordinated fashion to stimulation both by cold and by pressure (fig. 26). No indication could be found of either the true primary phase or the secondary phase in the melanophore reaction. The paleness of the embryos and young larvae in a dark room shows a primary phase reaction; but the fact that the melanophores of embryos and larvae of this stage contracted over a white background and expanded over a black background indicates the secondary phase.

What is the interpretation of these data? The adoption of the stellate condition by the melanophores of the majority of embryos regardless of their background or of the presence or the absence of light up to a time just previous to hatching shows these pigment bodies to be independent of any coordinating system. That they can respond to a stimulus is clearly shown by their action when subjected to cold and to pressure. Since all melanophores over the bodies of these young embryos were stimulated perhaps equally by both cold and pressure, it is believed that their response is an independent one and does not depend upon any coordinating agency. In contrast, however, these embryos show definitely just a few hours before hatching that their melanophores are coordinated, thus giving some evidence of an intervening agency at this time. The fact that both the eyeless and the normal embryos respond in the same way to the same background demonstrates clearly that this is some agency other than the eyes. It may be argued that the eyes of these embryos and larvae are not functional at this stage in development. The same results, however, are obtained from the hatched eyeless and normal larvae when the normal ones have very well-formed eyes. As additional proof, cited previously in this report, larvae still older than those just hatched give no visible response to white or to black backgrounds. The sudden appearance of this coordinating agency just previous to the hatching of these embryos, together with evidences of its disappearance soon after the larvae are hatched, indicates that it is some agency in the capsule. That a chemical change of some nature does take place within the capsule just before the embryos hatch is indicated by the definite weakening of the wall of the capsule at this time. This chemical change could account for the melanophore reaction herein recorded. The cessation of the conspicuous melanophore behavior soon after the larvae hatch gives further support to this view. The writer believes this agency to be a hormone. The proof of the presence of this agency is more real than is its identification, however. The absence of change in the color pattern of the adult fish might possibly indicate that the ontogeny of this animal has some bearing on the peculiar melanophore reactions of its embryos and larvae.

Summary and Conclusions

1. Melanin granules migrate within the melanophores of *Pomacentrus* embryos as soon as these pigmentary bodies are completely formed.
2. The melanophores of embryos a few hours of age contract in response to pressure applied with forceps and to a temperature of about 8° C.
3. The melanophores of a majority of developing embryos, from their beginning up to a few hours before hatching, are found to be in a stellate state regardless of whether these young are over a white or a black background or are in total darkness.
4. A few hours before hatching, the melanophores contract when the embryos are over a white background, expand when they are over a black background, and contract when they are in total darkness. This response continues in these young on through the hatching period and for a few hours after hatching.
5. Larvae of two or more days after hatching do not show any conclusive response to different backgrounds or to the absence of light.
6. The eyes of these young fish are believed to have no function in controlling their melanophore responses.
7. The evidence is in favor of the release of a hormone within the capsule just before the embryos hatch. This agency either permits or directly causes the melanophores to respond to various environments.

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PLATES

All illustrations in plates 1 to 4 are from photomicrographs.

PLATE 1

FIG. 1. A very early stage of the embryo. The developing embryo is shown on the right side of the illustration. × app. 200.

FIG. 2. Embryo showing the first appearance of melanophores. Note these bodies above the eye and near the heart. × app. 200.

FIG. 3. Same stage as in figure 2. Note the melanophores above the eyes. × app. 200.

FIG. 4. Same stage as in figure 2, showing the melanophores on the yolk as well as on the head. × app. 200.

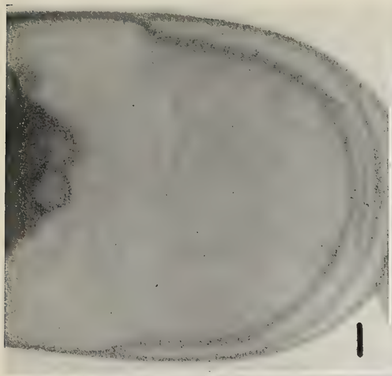
FIG. 5. Embryo just before hatching, showing the distribution of melanophores over its body as well as those on the yolk. These melanophores were contracted as a result of handling the embryo while removing its capsule from the substratum. × app. 200.

FIG. 6. Hatched larva, showing the distribution of its chromatophores at this stage in development. The chromatophores in the anterior part of the dorsal fin are yellow, while those in the posterior part of this fin and those in the ventral fin are brown. Most other color bodies are black. × app. 130.

FIG. 7. A mid-part of the body of a larva at a higher magnification than in figure 6, showing the contracted yellow chromatophores in the dorsal fin and the expanded brown chromatophores in the dorsal and in the ventral fin. Photographed at a stage of development four days later than that shown in figure 1. Subjected to a white background. × app. 200.

FIG. 8. A larva showing the melanophores migrating to the dorsal and ventral fins. × app. 200.

FIG. 9. A larva from the same batch of young as the one shown in figure 7, but subjected to a black background. × app. 200.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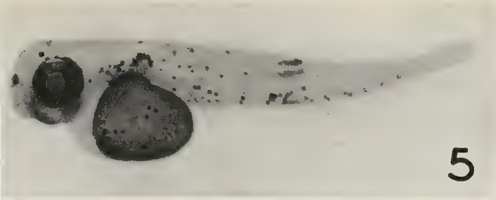
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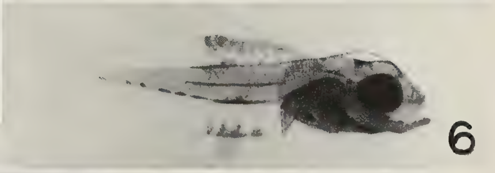
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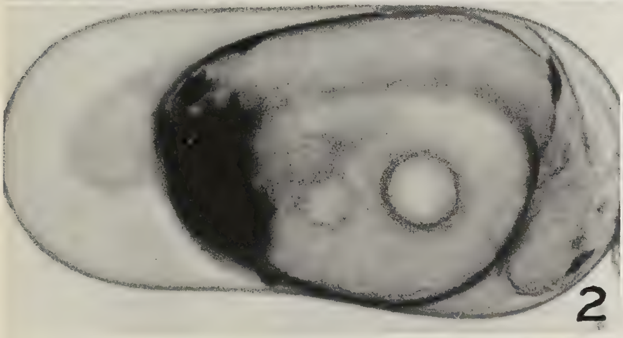
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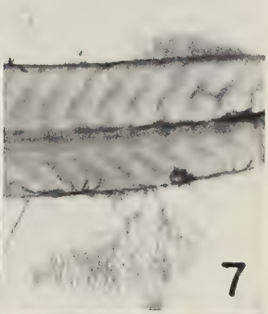
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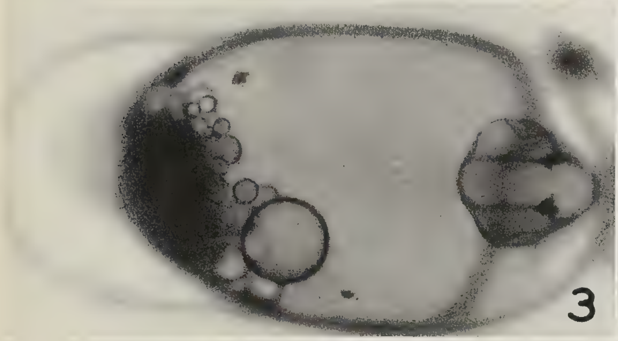
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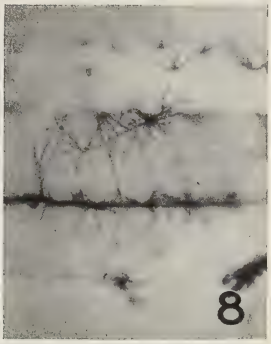
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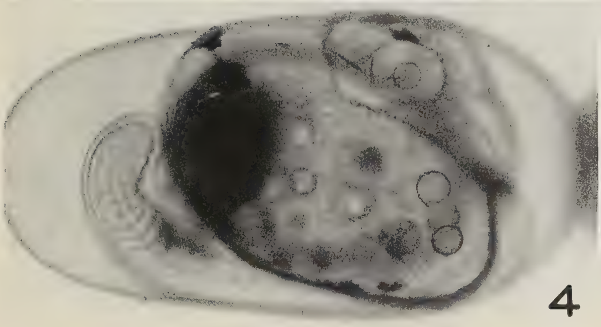
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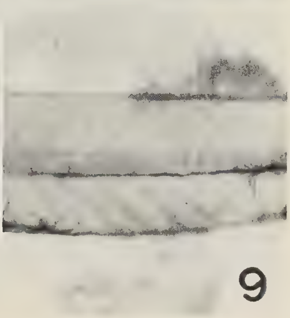
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PLATE 2

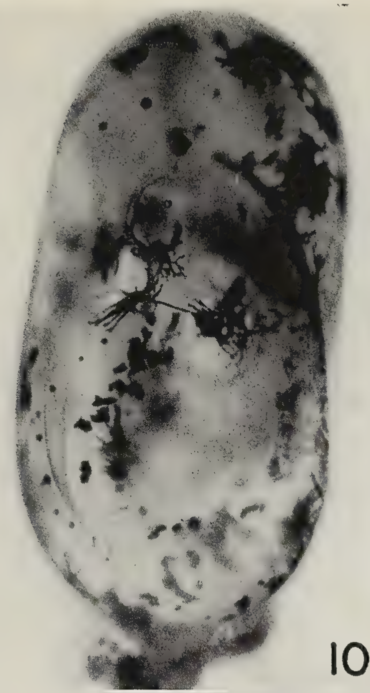
All figures × app. 200

FIG. 10. Embryo just before hatching, without eyes and kept over a white background. Melanophores are contracted.

FIG. 11. Embryo in the same stage of development as that shown in figure 10, but with eyes and kept over a white background. Melanophores are contracted.

FIG. 12. Embryo in the same stage of development as that shown in figure 10, but without eyes and kept over a black background. Most of the melanophores are expanded.

FIG. 13. Embryo in the same stage as that shown in figure 10, but with eyes and kept over a black background. Melanophores are expanded.



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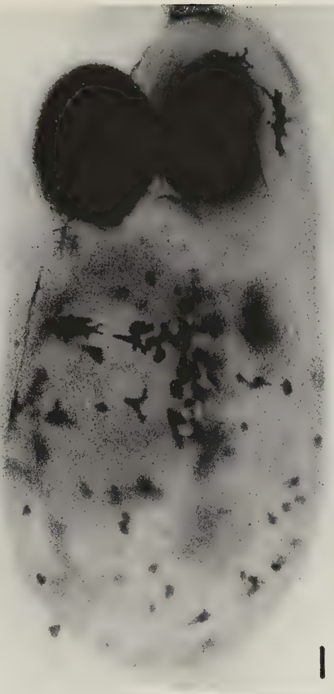

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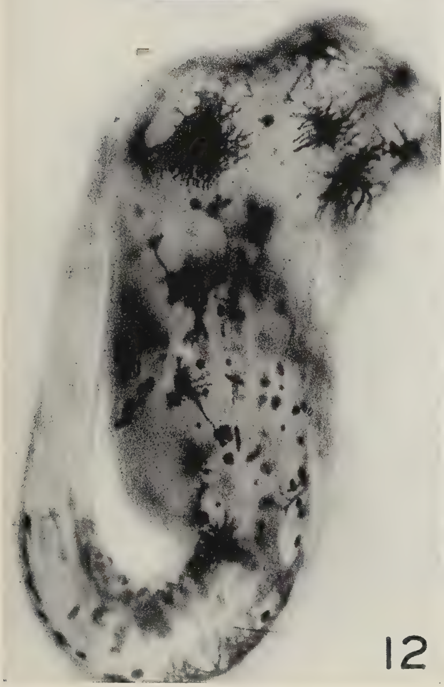

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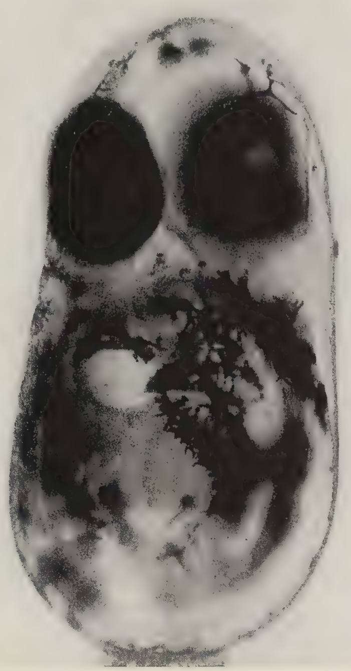

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Plate 3

All figures × app. 200

Fig. 14. A larva immediately after hatching. Kept in a dark room during its development, starting with the stage shown in figure 1. Melanophores are contracted.

Fig. 15. A larva in a stage later than that shown in figure 14. Subjected to a black background and showing expanded melanophores on its trunk region and contracted ones on its tail region.

Fig. 16. A larva in the same stage as that shown in figure 15, showing the effects of cutting the lateral line. The cut is shown in the middle of the photograph, where the melanophores are slightly contracted. The other melanophores are expanded. Photographed five hours after the cut had been made.

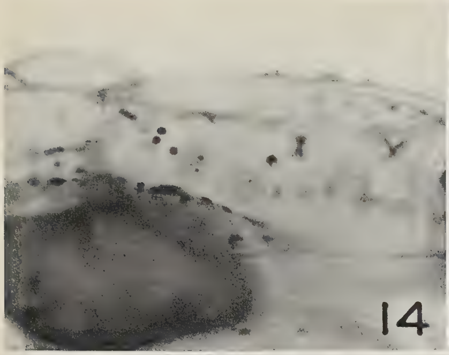
Fig. 17. A larva from the same batch of embryos as that shown in figure 14. Photographed at a stage of development a few hours later than that shown in figure 14. Melanophores are expanded.

Fig. 18. A larva just after hatching subjected to a black background. Note the expanded melanophores and the few contracted ones.

Fig. 19. A larva from the same batch of young as the one shown in figure 18. Subjected to a white background. Most of the melanophores are contracted.

Fig. 20. A larva of a stage later than that shown in figure 19. Without eyes and subjected to a black background.

Fig. 21. A larva of the same batch and the same stage as that shown in figure 20. With eyes and subjected to a black background. Note the maximal expansion of the melanophores, which are similar to those shown in figure 20.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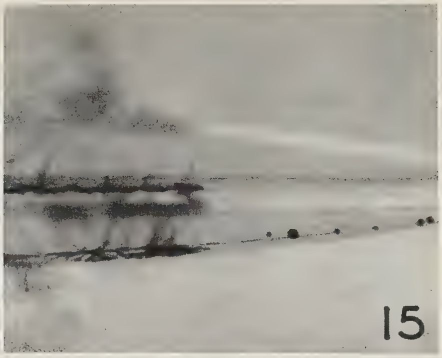
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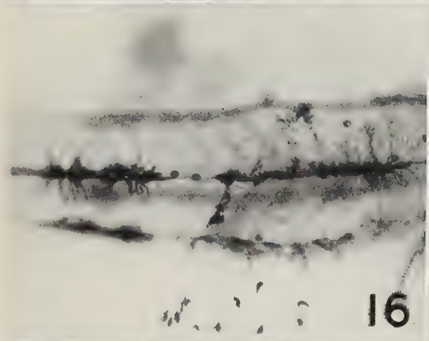
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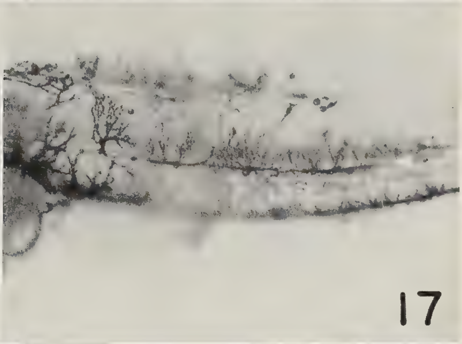
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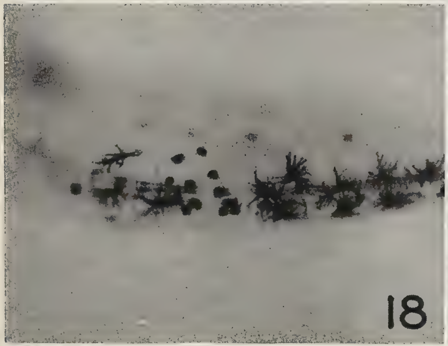
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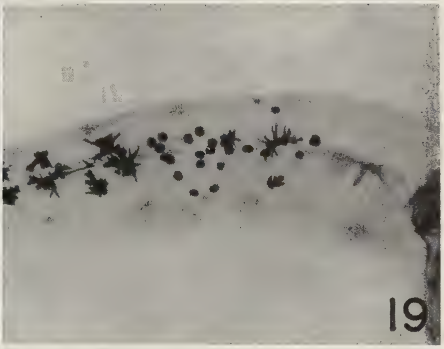
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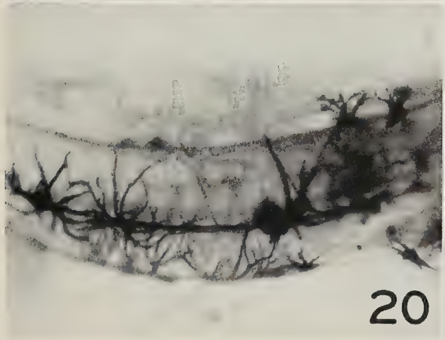
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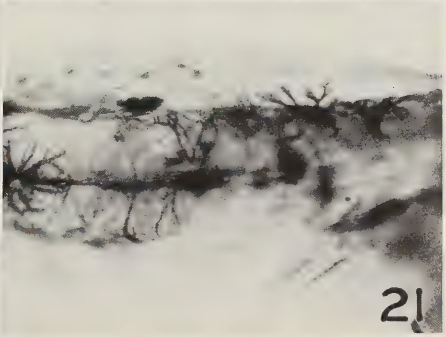
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PLATE 4

FIG. 22. Embryo just before hatching, kept in a dark room during its development from the stage shown in figure 1. Note the maximally contracted melanophores. × app. 200.

FIG. 23. Embryos subjected to a black background. Note the slightly expanded melanophores. × app. 130.

FIG. 24. Embryo from the same batch as that shown in figure 22, but photographed a few minutes earlier. Most of the melanophores are expanded. × app. 200.

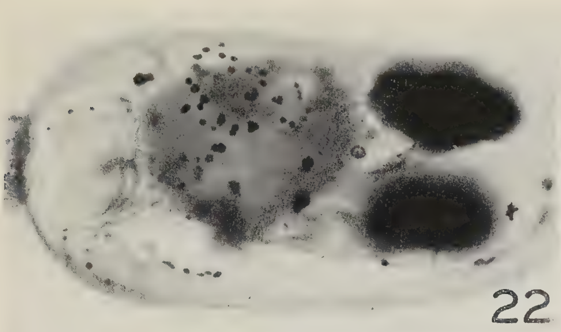
FIG. 25. Embryos from the same batch as those shown in figure 23, but kept in a dark room. Note the slightly contracted melanophores. × app. 130.

FIG. 26. Embryo photographed 24 hours after it had been squeezed with forceps. Contraction of the melanophores was due to this pressure stimulus. × app. 200.

FIG. 27. Embryos from the same batch as those shown in figure 23, but subjected to a white background. Photographed at the same time as those in figures 23 and 25. × app. 130.

FIG. 28. Embryo from the same batch as that shown in figure 26. This embryo was not squeezed and shows the stellate melanophores. × app. 200.

FIG. 29. Embryos from the same batch as those shown in figure 23, but kept in a clear finger bowl on a light-colored table. × app. 130.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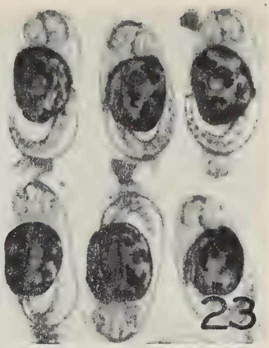
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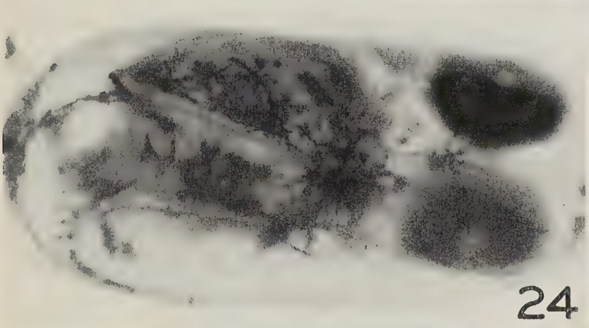
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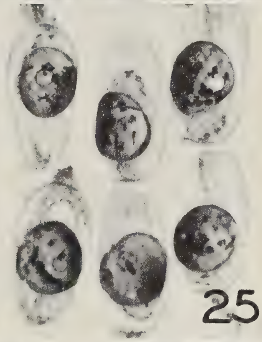
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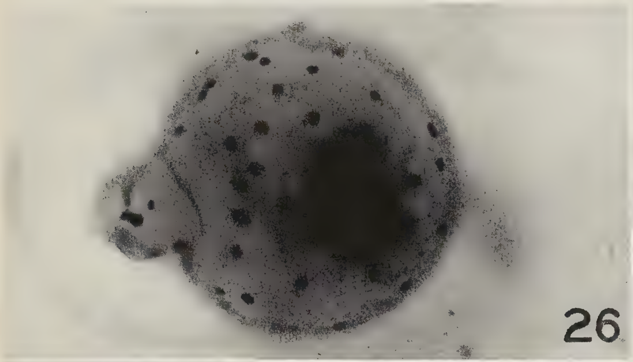
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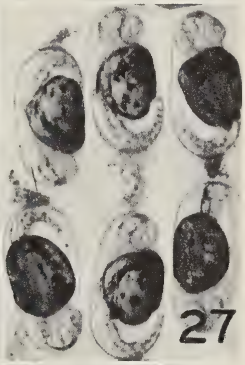
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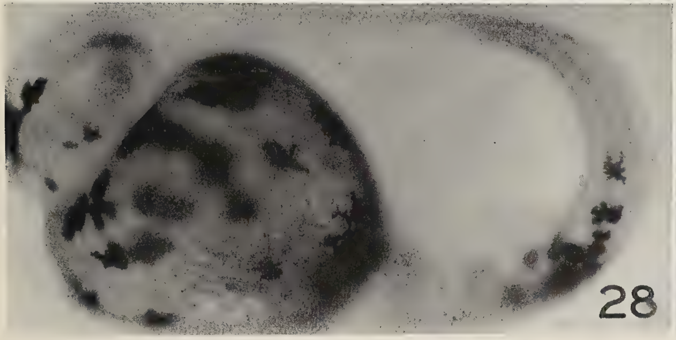
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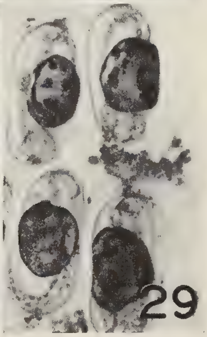
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PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

XIII

Some Ciliates from Tortugas

By W. E. Bullington

Randolph-Macon College

With one plate and twenty-five text figures

[ISSUED SEPTEMBER 14, 1940]

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Some Ciliates from Tortugas

Introduction

During the summers of 1930, 1931, and 1935, while the author was making a special study of spiraling in certain species of ciliates at Tortugas, there appeared in his cultures from time to time many other species which seemed to be new or but little known. Some of these had previously been seen elsewhere, but most had never been seen before. They were characterized by an amazing display of color, in which shades of yellow and red predominated. There seemed also to be a greater abundance of species of the order Hypotricha.

These ciliates were studied whenever they appeared, in so far as time permitted. Characteristic sketches were made of each, and as much information as possible was obtained regarding size, form, structure, color, etc. Some of these ciliates appeared only once and were gone before a satisfactory study could be made. In many cases, the information obtained was so incomplete that the species could not be identified.

During the summer of 1939, a special effort was made to relocate these species in order to complete the missing information. There are now fifteen species, either new to science or but little known, about which it is believed sufficient information is available to justify their description or redescription. Five of the fifteen species have previously been described, but none of them is well known. One of the five, *Condylostoma magnum* Spiegel 1926, belongs to the genus which is probably most common and is generally recognized. The other ten species are being described as new. One of these belongs to a genus which includes characteristics of both the Heterotricha and the Hypotricha, and has been seen only once before. One new genus is being created.

The author wishes to express his appreciation to the Carnegie Institution of Washington for assistance in making possible this study at the Tortugas Laboratory. Microphotographs, made chiefly as an aid to drawing, were taken with equipment purchased with funds provided in grants by the Virginia Academy of Science. The author is indebted also to the following: Dr. J. B. Haley, for his kindness in checking scientific names; Mrs. J. B. Haley, librarian at Randolph-Macon College, for her generous help in securing by loan from other libraries the literature not available at this library; and the Interlibrary Loan Division of the following libraries for their cooperation in making their stacks available through the Randolph-Macon College Library: the Library of Congress, the Library of the Surgeon General's Office, U. S. War Department, the Library of the U. S. Department of Agriculture, the Library of the U. S. Bureau of Fisheries, the Library of the American Museum of Natural History, the Library of the Smithsonian Institution, and the Johns Hopkins University Library.

Source of Material

The ciliates at Tortugas appear, in general, somewhat more transitory than those found elsewhere. Many of them suddenly appear and as suddenly disappear. This is of course to be expected, to some extent, and may be due to several causes, e.g. temperature of the water, lack of food, etc.

Not many of these ciliates have been found near the surface of the water; most have come from near the bottom. This is due in part perhaps to the higher temperature at the surface, but probably chiefly to the fact that very little food is found there. Food is found on algae and decaying material, which is usually on or near the bottom, scattered over the coral rock and in quiet pools. The two pools at either end of Loggerhead Key, formed by the shifting sands at the tips and having on the bottom great masses of decaying turtle grass brought there by the waves, are excellent sources of material. The moat at Fort Jefferson is also an excellent source. The growth of algae along the walls and near the bottom usually contains several species. It was from this material that *Nassula gigantea* n. sp. came. A few ciliates have been found on floating algae at the dock at Fort Jefferson. From the ponds on Bush Key have come certain species of *Condylostoma* and *Frontonia*. But probably the richest, surest source of material, the one in which ciliates are found in greatest abundance and variety, is the tip of Long Key. Here are great masses of several species of living algae, attached to the coral rocks, and although the water at this point is only 6 to 8 inches deep or even less at low tide, and becomes in consequence extremely warm, cultures of this material never fail to contain several species of ciliates. One or more species of *Frontonia* are practically always found there.

Descriptions of Species

Nassula gigantea n. sp.

This species was found several times on algae in the bottom of the moat on the south side of Fort Jefferson, during the summer of 1939. It had previously been seen during the summer of 1931, but was not then recognized as a ciliate.

Description

Form. Elongated, the two ends rounded, but the right[1] side of the posterior end less rounded; sometimes somewhat irregular in outline, but usually cylindrical, particularly in the posterior three-fourths; sometimes slightly flattened in the anterior fourth, which projects out as a sort of rounded knob to the animal's left (fig. 1*A, B*), somewhat as is shown in *Nassula aurea* Ehrbg. 1833 (according to Kahl) and *N. brunnea* Fabre-Dom. 1885; slightly constricted immediately posterior to this projection.

Color. A very dark brown, so dense it is almost black.

Size. Length (28 measurements) 260 to 624 microns, average 451; width (21 measurements) 139 to 260 microns, average 181. Length about 2 to 2.4 times the width.

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[1] The terms "right" and "left" as used in this paper, unless otherwise stated, refer to the right and left of the organism.

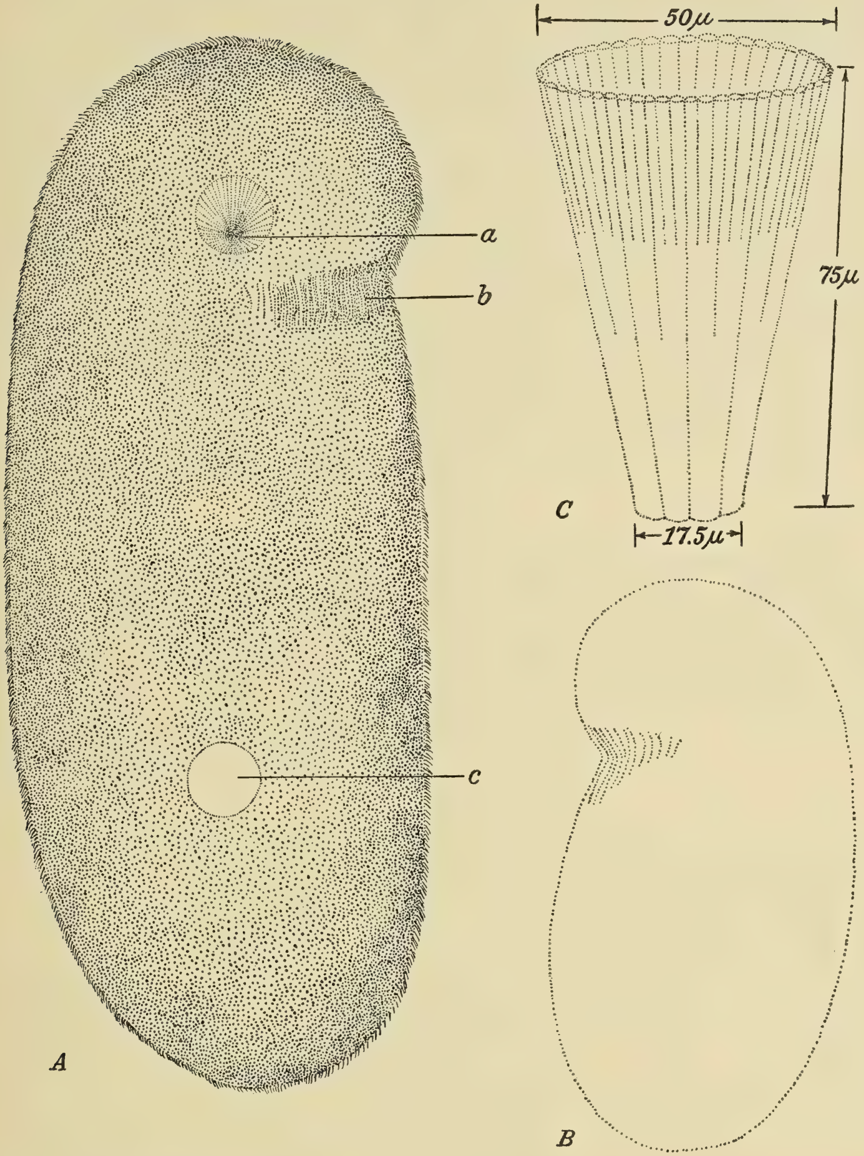

Fig. 1. *Nassula gigantea* n. sp.

A. Ventral view, × 300, showing structure. *a*, mouth, showing pharyngeal rods; *b*, constricted necklike region; *c*, contractile vacuole.

B. Dorsal view, showing general form.

C. Pharyngeal basket, greatly enlarged, showing general form and size, and the nature of the pharyngeal rods.

Mouth and pharynx. Mouth more or less circular, located near the mid-line (fig. 1*A*), about ⅕ the length of the body from the anterior end. The pharyngeal rods can be seen through the mouth and appear more or less like spokes in a wheel (fig. 1*A*); these gave the first clue to the identity of this species. A perfect view of the entire pharyngeal basket was furnished by one individual which went to pieces under the microscope. The pharyngeal basket came out of the body intact and remained so for some time. It was smaller at the inner end, larger at the outer, measuring 50 microns in diameter at the larger end, 17.5 microns at the smaller end, and 75 microns in length. It was made up of about 48 rods at the outer end, 24 being counted on one side. See figure 1*C*, a greatly enlarged view, with the 10 × ocular and the 4-mm. objective. The pharyngeal rods point perpendicularly into the protoplasm from the mouth, rather than at an angle to the right or left, as in most other species. Each individual rod is larger at the inner end and is divided into four rods at the outer end. There are no rings of any sort around the pharyngeal basket (fig. 1*A*).

Contractile vacuole. Only one has been seen, located in the posterior third of the body (fig. 1*A*).

Nucleus. Not seen.

Spiraling. This species does not often swim freely, but spends most of its time moving about over the masses of débris. Twenty-seven spirals were secured, however, by isolating a few individuals in a watch glass, removing as much of the débris as possible, and adding clean sea water. Even after this, most of the swimming was toward or away from the surface film. The 27 spirals which were observed and measured were all to the left and averaged about 1232 microns in length. Since some of these were at a slight angle to the surface film rather than horizontal, it is thought the length of spiral may average somewhat more than this.

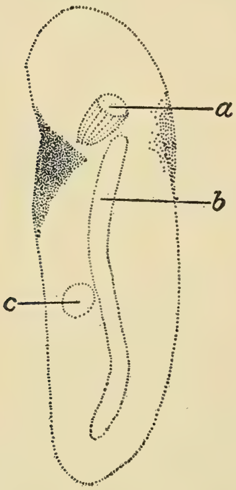

FIG. 2. *Nassula brunnea* Fabre-Dom. Redrawn from Fabre-Dom., to show general form. *a,* mouth; *b,* nucleus; *c,* contractile vacuole.

Discussion

Reasons for identifying this as a species of *Nassula:* (1) presence of a pharynx lined with pharyngeal rods; (2) subterminal position of the mouth, about ⅕ the length of the body from the anterior end. These are important *Nassula* characteristics.

Reasons for describing as a new species: (1) This is the largest species of *Nassula* yet described. Kahl (1935) lists two species of *Nassula* having a length of as much as 300 microns: *Nassula elegans* Ehrbg. 1833, measuring 200 to 300 microns, and *N. brunnea* Fabre-Dom. 1885, measuring 250 to 300 microns. The species here described exceeds them by almost twice their length and approximately one and one-half times their width. (2) The color of *Nassula gigantea* differs from that of any previously described species in that it is more nearly black. *Nassula brunnea,* which most nearly approaches it in color, is very light brown in comparison. (3) *Nassula gigantea* differs from *N. brunnea,* which it most closely resembles in form, in that the anterior projection is to the left in the former, and to the right in the latter (fig. 2). (4) *Nassula gigantea* has its contractile vacuole in the posterior third of the body, *N. brunnea* near the middle.

Diagnosis

Nassula gigantea n. sp. is an extremely large species, measuring approximately 451 × 181 microns; color very dark brown, almost black; anterior third of body projects somewhat to the left; slight constriction immediately posterior to this projection; pharynx somewhat funnel-shaped, larger at outer end than at inner, measuring about 75 microns in length, lined with about 48 to 50 rodlike teeth (at outer end; approximately 12 at inner end); one contractile vacuole near posterior end; swims in left spirals.

Ophryoglena frontonia n. sp.

During the summer of 1930, in the small blaster-out pool on East Key from which *Frontonia schaefferi* Bullington 1939 came, a very small ciliate was found which was at first identified as a species of *Frontonia.* This pool being dry during the summer of 1931, no more were found there, but others were found in some of Dr. D. L. Hopkins' discarded ameba cultures, collected some time previously from the moat around Fort Jefferson.

Description

Form. In free swimming this species appears distinctly foot-shaped, being widest at the anterior end and narrowed at the posterior end (fig. 3*A*), a shape typical for certain species of *Frontonia,* and for this reason it was at first thought to be a species of *Frontonia.* But individuals isolated and examined under a cover glass through the 4-mm. objective and the 10 × ocular were found to be pointed instead of rounded at the anterior end, wider and more rounded at the posterior end (fig. 3*B*).

Color. Clear or slightly brown, not different from most species of *Frontonia* except that perhaps the posterior third of the body is darker and browner than in *Frontonia.* This, however, may merely have indicated massing of food vacuoles in this area.

Size. Length (40 measurements) 66 to 132 microns, average 92; width (33 measurements) 48 to 66 microns, average 50. Width ½ to ¾ the length.

Contractile vacuole. Not seen.

Mouth. Pocket-shaped, without oral groove.

Striations. Body very coarsely striated, both longitudinally and transversely. These striations, however, are not caused by cilia, but by the pimple-like projections of the trichocysts, as in *Frontonia.* Both longitudinal and transverse striations can be clearly seen (4-mm. objective and 10 × ocular) over the surface of the body, where they form little squares (fig. 3*B*). But on the edge of the body these squares appear as serrations, as in *Frontonia* (Bullington, 1939), except that those in *Ophryoglena* seem to be somewhat more prominent.

Nucleus. Long and somewhat dumbbell- or club-shaped (fig. 3*B–G*), usually bent around in the body as indicated in the drawings. The nucleus can be seen in living individuals, but stained preparations were used as a check.

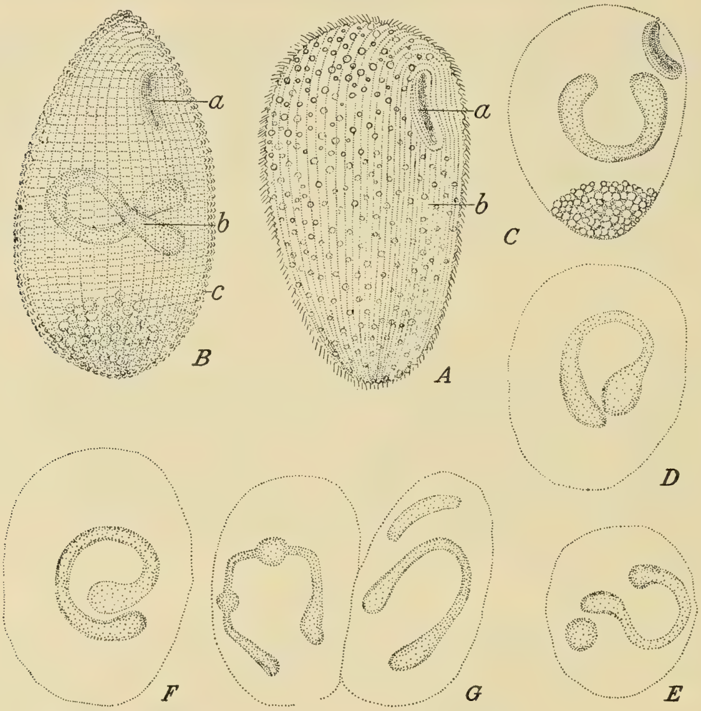

Fig. 3. *Ophryoglena frontonia* n. sp.

A. Ventral side view, × 500, showing general form like that of *Frontonia. a,* mouth; *b,* striations.

B. Ventral side view, × 500, showing form variation, and striations formed by trichocysts. *a,* mouth; *b,* nucleus; *c,* serrations or pimple-like projections above trichocysts.

C. Outline drawing, × 375, showing general form, nucleus, and posterior vacuolated structure.

D, E, F. Outline drawings showing the nucleus.

G. Conjugating individuals, showing the nucleus.

Spiraling. In forward swimming all spiraling observed has been distinctly to the left, the spirals averaging 817 microns in length (50 measurements, 322 complete spirals, varying from 500 to 1666 microns). Average speed of swimming, 1862 microns per second (27 measurements, varying from 1309 to 3333 microns per second) (fig. 4).

All backward swimming has been uniformly to the right, but no measurements either of speed or of size of spirals in backward swimming have been possible. The direction, however, is the same as that found in all species of *Paramecium* and *Frontonia* (Bullington, 1930, 1931, 1939).

Discussion

This species was at first thought to be a frontonian, until the mouth was seen. The genus *Frontonia* is characterized by a broadswordlike mouth, with a long, slender groove extending posteriorly from the lower side of the mouth. *Ophryoglena frontonia* has a pocket-shaped mouth (fig. 3) and no oral groove of any sort. These two characteristics definitely separate the two genera.

The macronucleus in *Frontonia* is a large oval body; in *Ophryoglena frontonia* it is very much elongated and enlarged into knoblike structures at the ends, and folded over on itself. No species seems to have been described with these characteristics. This species is also smaller than any *Frontonia* now known. Even *Frontonia schaefferi*, the smallest *Frontonia* reported to date, measures approximately 120 × 66 microns, as compared with 92 × 50 for *Ophryoglena frontonia*.

Ophryoglena viridis Penard is of about the same size (80 microns long) and general shape, but it is green and has a circular or ring-shaped mouth.

Ophryoglena maligna Penard is of about the same size, but it has an eye spot and is entoparasitic on the larger *O. flava*. *Ophryoglena frontonia* has no eye spot, nor is it entoparasitic.

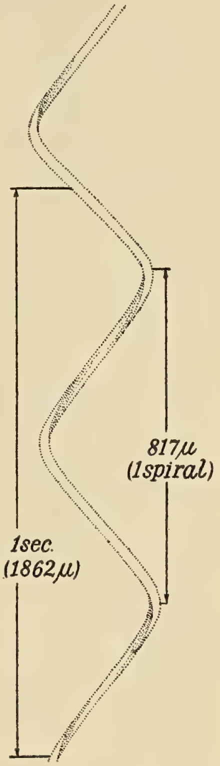

FIG. 4. Spiral path of *Ophryoglena frontonia*, × app. 50.

Diagnosis

Ophryoglena frontonia n. sp. is a small ciliate, sometimes foot-shaped, somewhat like *Frontonia,* sometimes pointed anteriorly and wider posteriorly; measures approximately 92 × 50 microns; clear or slightly brown in color; mouth pocket-shaped, no posterior groove; coarsely striated longitudinally and transversely, the crossing of these lines forming little squares on the surface of the body, serrations on the edge; macronucleus dumbbell-shaped, folded upon itself; swims in left spirals forward, right spirals backward.

Condylostoma magnum Spiegel 1926

This species was found in standing pools on Bush Key Reef at extreme low tide during the summers of 1930, 1931, and 1935.

Description

Form. Condylostoma magnum Spiegel, as found at Tortugas and identified by the author, is extremely long and wide, wider in proportion to length than the individuals Spiegel observed. It is widest centrally, narrowed and rather pointed posteriorly, somewhat constricted along the peristome in the region of the mouth, and slightly flared out again at the extreme anterior end. The body is extremely flexible and may be extended and contracted within very wide limits (fig. 5).

Color. Pinkish.

Size. Length (42 measurements) 572 to 1649 microns, average approximately 960; width (27 measurements) 129 to 301 microns, average approximately 191. Width about ⅕ the length. Spiegel reported a variation of from 600 to 900 microns in length, and from 90 to 120 microns in width. The individuals which he studied were therefore much narrower than those reported here. This culture was studied for several days to see whether some individuals might not at times assume a more slender form, particularly when food became scarce, but no change could be detected.

Striations. This species is finely ciliated and longitudinally striated, 40 to 50 striations being counted on the upper surface (Spiegel reported 40). These striations are made up of alternating rows of fine cilia and narrow bands of fine granules, about one row of granules to each band. The bands are darker, and the lines of cilia are lighter (see fig. 6).

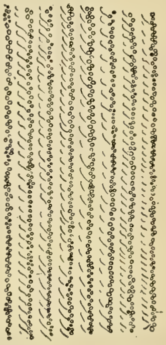

Fig. 6. *Condylostoma magnum* Spiegel 1926. Section of body surface, greatly enlarged, to show striations.

Mouth and peristome. Mouth at extreme

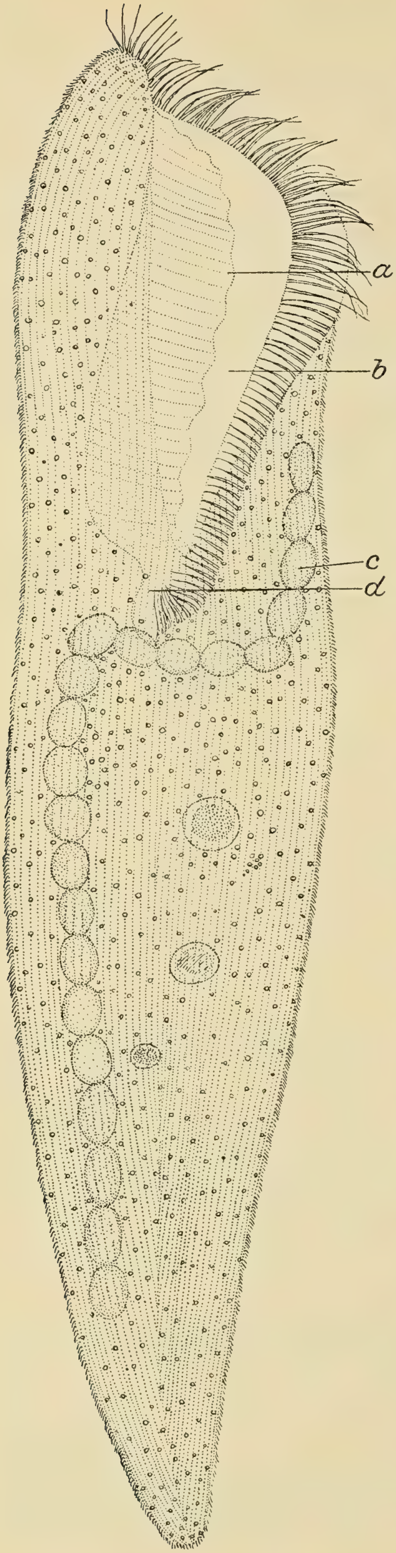

Fig. 5. *Condylostoma magnum* Spiegel 1926. Ventral view, × 200, showing general form and structure. *a,* undulating membranes; *b,* peristome; *c,* nucleus; *d,* mouth.

posterior end of the peristome (fig. 5), approximately 207.5 microns from the anterior end. This is about ¼ to ⅓ the body length. In the characteristic sketches made at the time these animals were under observation, the peristome is shown extending fully ⅓ the length of the body, and is wider and more prominent than has been shown in previous drawings (fig. 5).

Nucleus. Long and moniliform, running from near the posterior end up the right side and across the anterior peristomial area. In one individual it extended across the mouth and up a short distance on the left side. It is composed of twenty or more beads; one individual had 22.

Spiraling. This species swims in left spirals, which have measured in length, on the average (7 measurements), about 1478 microns per spiral.

Discussion

This species is identified here as *Condylostoma magnum* Spiegel, but there are some differences to which it seems necessary to call attention. The chief difference is that of size. The narrowest individuals seen by the author were from 1 to 3 times wider than the widest ones reported by Spiegel. The shortest ones were somewhat under his lower limit (600 microns), and the longest almost twice his upper limit (900 microns).

There is very little, if any, difference in the number of longitudinal striations, but the peristome is probably a little more prominent in the form described here. The posterior extremity of the individuals seen by Kahl (1932) is more pointed than in the form described here.

Diagnosis

According to the present observations, *Condylostoma magnum* Spiegel is extremely long and wide, measuring approximately 960 × 191 microns; length about 4 to 5 times the width; widest centrally, pointed posteriorly, but not so elongated as indicated by Spiegel (1926) and Kahl (1932); constricted in the mouth area, flared out again somewhat at the anterior extremity; 40 to 50 longitudinal striations on the upper surface; peristome large; mouth, at end of peristome, about ¼ to ⅓ the body length from the anterior end; macronucleus long, moniliform, 20 or more beads; swims in left spirals.

Condylostoma granulosum n. sp.

Condylostoma granulosum was found in the pools on Bush Key during the summers of 1930 and 1935, in cultures with *C. magnum*.

Description

Form. Somewhat elongated, but with a greater width in proportion to length than in *C. magnum;* somewhat flattened, not cylindrical, especially in the anterior half of the body; widest centrally, gradually narrowed toward the two extremities. Posterior end rather bluntly rounded, anterior end truncate; left side, in the region of the mouth, constricted (concave); below this point, left side rather prominently and convexly curved all the way to the extreme posterior end. Right side convexly curved its entire length from the anterior to the posterior extremities; this gives a distinct left curve to the anterior end of the body (fig. 7).

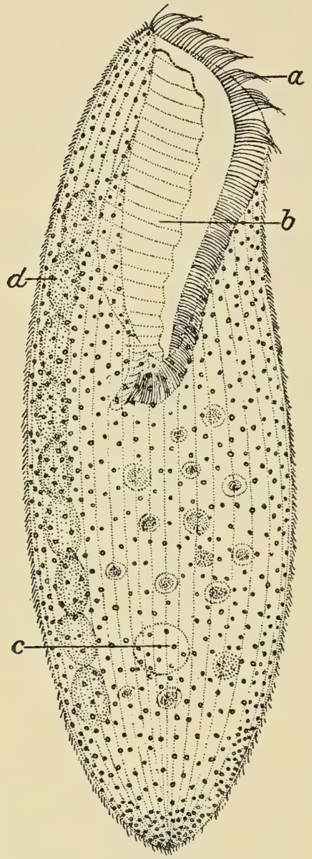

Fig. 7. *Condylostoma granulosum* n. sp. Ventral view, × 400, showing general form and structure. *a,* peristomial cilia; *b,* undulating membrane; *c,* contractile vacuole; *d,* nucleus.

Color. Slightly yellowish.

Size. Length (18 measurements) 141 to 588 microns, average 292; width (13 measurements) 66 to 196 microns, average 93. Width about ⅓ the length.

Contractile vacuole. Only one has been seen, located in the posterior third of the body (fig. 7).

Nucleus. Moniliform, composed of 8 to 9 beads, lying usually on the animal's right side (fig. 7). The individual beads are larger than those in *C. magnum.*

Oral cilia. Very prominent, measuring about 17.5 microns in length.

Striations. The body is completely ciliated but coarsely and longitudinally striated (fig. 8). Sixteen striations were counted on the upper surface.

Spiraling. Individuals swim in left spirals, average speed (4 measurements) 1733 microns per second. They also move around in circles to their left while swimming in normal left spirals. This type of swimming is identical with that found in certain species of *Frontonia* (Bullington, 1939).

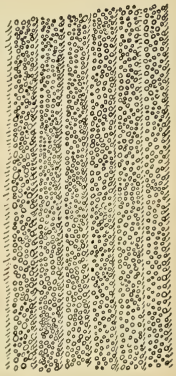

Fig. 8. *Condylostoma granulosum* n. sp. Section of body surface, greatly enlarged, showing lines of cilia and the wide bands of granules separating them.

Discussion

This species appears to be the same one found by the author at Cold Spring Harbor during the summer of 1923 (in material from the brackish pond back of Jones' boathouse) and, for lack of sufficient information, identified as *C. patens.* See *Condylostoma (Trichoda) patens* (O. F. Müller 1786) Dujardin 1841. The individuals were of practically the same size as those at Tortugas (291 to 595 microns in length by 90 to 120 in width), and had the same general form, the same number of striations, equally prominent oral cilia, and a body similarly dorsoventrally flattened. But *C. patens* has a cylindrical body, and measures only

200 microns in length (C. & L.), 305 to 495 (Maupas), 376 to 564 (Stein), 400 × 100 (Calkins), or up to 500 (Kahl, 1932). Owing to this wide variation, it is quite possible that length alone is an insufficient basis for drawing a clear line of demarcation between *C. patens* and *C. granulosum*. The former is cylindrical, however, instead of flattened, and the body is narrower; and, most important of all, it has several vacuoles (8, according to C. & L.) located along the left side, or (according to Kent) several vesicles forming a part of a vesicular canal. Kahl shows a posterior vacuole with a long extension up one side. All investigators seem to agree on more than one vacuole, which clearly separates this species from *C. granulosum* with its one vacuole.

Condylostoma arenarium Spiegel 1926 might be considered comparable in general shape, being wider in the middle and narrowed toward each extremity, and it has about the same number of striations (14 to 15); but it is cylindrical, not flattened, and length is much greater in proportion to width; its peristome is smaller and less prominent, and its nucleus is composed of 13 to 16 beads instead of 8 to 9 as in *C. granulosum*.

Condylostoma rugosum Kahl 1932 has about the same number of striations (10 to 16), but is more cylindrical, very slender, and only 300 to 500 microns in length.

In *C. magnum,* the nucleus has a larger number of beads, of smaller size; and the lines of cilia are separated by narrow bands of one row of granules, instead of by broad bands as in *C. granulosum*.

Diagnosis

Condylostoma granulosum n. sp. is somewhat elongated, more or less dorsoventrally flattened, not cylindrical, bluntly rounded or slightly pointed at the posterior extremity, widest centrally, concave on the left side along the peristome, convex below; right side convex its entire length and causing the body to curve to the animal's left anteriorly; color generally slightly yellowish; contractile vacuole, one, located in the posterior third of the body; body longitudinally and coarsely striated, about 16 striations over upper surface, lines of cilia separated by relatively broad bands of granules; nucleus moniliform, composed of about 8 to 9 beads; average size about 292 × 93 microns, but may vary from 141 × 66 to 588 × 196 microns; width about ⅓ the length.

Condylostoma minutum n. sp.

During the summer of 1930 and again in 1935 the author found in some of his cultures a very small species of *Condylostoma* which differs from others, particularly in size, but no record was made as to the source of the material.

Description

Form. Very small, with a narrow, cylindrical, and elongated body, resembling *C. arenarium* except that it is much smaller, more slender in relation to length, and not so pointed at the posterior extremity. The two sides of the anterior half of the body are straight and practically parallel, the body being only slightly wider, if any, centrally. The posterior half tapers gradually to a bluntly rounded posterior extremity, and, in some individuals, is curved sharply in a hooklike projection to the animal's left (fig. 9).

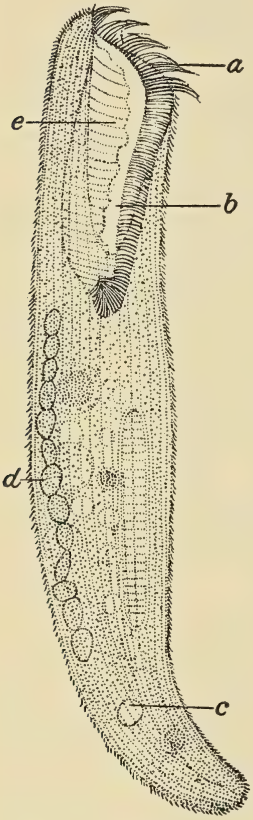

FIG. 9. *Condylostoma minutum* n. sp. Ventral view, × 400, showing general form, structure, etc. *a*, peristomial cilia; *b*, peristome; *c*, contractile vacuole; *d*, nucleus; *e*, undulating membrane.

Color. Pinkish.

Size. Length (14 measurements) 199 to 264 microns, average 242; width (6 measurements) 33 to 88 microns, average 42. Length from 3 to 5 times greater than the width.

Striations. The body is finely and longitudinally striated (fig. 9). Sixteen striations were counted on the upper surface. This would mean probably 32 to 40 striations over the entire body (fig. 9), the same number as in *C. granulosum.*

Nucleus. Moniliform (determined in living individuals), and composed of about 13 beads.

Contractile vacuoles. Not seen.

Peristome. Relatively large, about ¼ the length of the body.

Spiraling. This species swims in left spirals, but it was not possible to determine the size of spirals or the speed of swimming.

Discussion

Condylostoma tardum Penard 1922, with a length of 180 to 250 microns, falls within the size range of this species, but has a nucleus of 3 globular parts and a somewhat spirally wound peristome. Moreover, there is some doubt in the mind of the author as to its being *Condylostoma. Condylostoma luteum* Kahl 1932 comes also within the size limits, but its shape is different, and it has an irregular contractile vacuole at the posterior end, and two spherical to oval nuclei. *Condylostoma vorticella,* another species comparable in size, is eliminated from consideration by its shape alone. *Condylostoma* sp.? Kahl 1935 is also of the same general size, but has a tail-like extension to the body, indicating that this is probably *C. caudatum* Lauterborn. *Condylostoma minutum* is not a small variety of *C. granulosum,* since *C. granulosum* is about ⅓ as wide as long, whereas *C. minutum* is only about ⅕ as wide as long; *C. minutum* is of almost the same width throughout, whereas *C. granulosum* is much wider centrally; the nucleus of *C. granulosum* is composed of 8 to 9 beads, that of *C. minutum* of about 13.

Diagnosis

Condylostoma minutum n. sp. is a very small, elongate, and very narrow species. Sides of anterior half practically parallel, cylindrical, only very slightly wider centrally if at all, bluntly rounded posteriorly; body finely and longitudinally striated, 16 striations counted on upper surface; average size 242 × 42 microns; nucleus moniliform, composed of about 13 beads; peristome about ¼ body length; swims in left spirals.

Need for a Special Study of Condylostoma

It is entirely too difficult at present to differentiate the species of *Condylostoma*. The entire genus needs a special study, and perhaps a redescription, or at least greater emphasis on the chief distinguishing features of the various species, in order that investigators who may not be particularly interested in the taxonomy of the group may more easily identify a species. The author hopes soon to be able to make such a study.

Peritromus tortugensis n. sp.

During the summer of 1930 the author found in a discarded ameba culture, collected earlier in the summer by Dr. D. L. Hopkins (exact source not known), a species of *Peritromus* which was apparently undescribed. During the summer of 1939 it was found again, more than once, this time in algal cultures from near the tip of Long Key at very low tide.

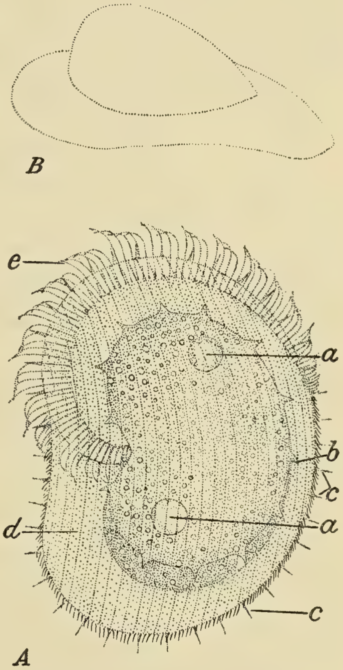

Fig. 10. *Peritromus tortugensis* n. sp.

A. Dorsal view, × 500, showing general structure, form, etc. *a*, nuclei; *b*, heavier central spine; *c*, lighter, more slender, outer spines; *d*, striations; *e*, oral cilia or styles.

B. Side view, outline only, showing depth and general shape.

Description

Form. Almost circular in outline as viewed from above, but slightly longer than wide (fig. 10*A*). In side view it is shaped very much like an old wide-brimmed hat (fig. 10*B*): the outer surrounding part of the body is flattened, bandlike, representing the brim of the hat; the inner central portion is raised above the brim and represents the crown. Calkins (1902) shows a very similar form for *P. emmae*. The body is very contractile, and contracts usually upon the slightest jar.

Color. Slightly yellowish, as in *P. emmae* (Calkins, 1902).

Size. Length (27 measurements) 70 to 147 microns, average approximately 100; width

(21 measurements) 53 to 86 microns, average approximately 74. The body is thus only slightly elongated, or practically circular (fig. 10*A*). Only one individual was found measuring as much as 147 microns in length. Three measured 129 microns each, but the greater number measured around 96 to 108 microns.

Nucleus. Two nuclei, one on the right side of the anterior extremity, the other on the left of the posterior extremity (fig. 10*A*), each measuring about 17.5 microns in diameter.

Cilia. The dorsal body surface is covered with fine cilia, which pass over the raised crown as well as the flattened brim, and are arranged in longitudinal lines or striations. Across the entire body at the widest point are 25 to 30 of these ciliary lines: from the right side to the mouth, 22 to 23, and from the mouth to the left side, 6 to 7. These ciliates were formerly classified as members of the Hypotricha, a group in which the cilia, very much modified, are limited to the ventral surface. Maupas seems to have been the first to discover that there were cilia on the dorsal side. These cilia, however, are not all of the same size, the oral cilia being longer and more prominent. This places *Peritromus* in the order Heterotricha. Kahl seems to have been the first so to classify the genus. Yet Kahl's drawings of his *Peritromus montanus* show cilia on the ventral surface only. The striations in *Peritromus tortugensis* have been clearly seen in surface view, but on the ventral surface no cilia other than the modified oral cilia (cirri) have actually been seen.

Spines. Peritromus tortugensis is characterized by a row of rather widely spaced, slender, and elongated spines projecting from the outer edge of the body slightly beyond the body cilia; and by larger spines on the central or crown portion of the body, relatively wide-based and sharply pointed, and projecting from the upper edge of the crown. Those on the outer edge are more slender and conspicuous. Neither set has been seen in side view.

Spiraling. Peritromus tortugensis swims in left spirals which measure approximately 278 × 109 microns (6 measurements of length and 4 of width), varying from 220 to 366 microns in length and from 88 to 132 in width (fig. 11). The speed is very slow, about 408 microns per second (2 measurements). This species swims very infrequently. When it is feeding over the bottom it is constantly turning, without spiraling, to its right side.

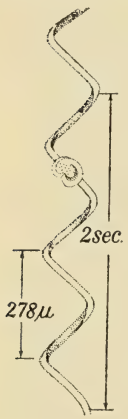

FIG. 11. *Peritromus tortugensis* n. sp. Spiral path, × 50, showing general length and width of spiral.

Discussion

This species corresponds to *P. emmae,* as described by Calkins (1902), in general form, yellowish color, and general size. But so far as is known, *P. emmae* has not been described with spines, whereas *P. tortugensis* has two sets of spines, as described above. Kirby (1934) shows similar spines on his *P. californicus,* with the apparent difference that the spines project up vertically from both the central raised crown and the flattened brim, whereas those on *P. tortugensis* project out laterally (horizontally). Moreover, Kirby's species is larger and more elongated than is *P. tortugensis.*

Peritromus faurei (P. emmae Faure-Frém. 1924) Kahl 1932 also has spines on both the central crown and the flattened brim, but here again the spines extend up over the dorsal surface and the body is very much elongated, more slender than that of *P. tortugensis,* and much larger. *Kerona ciliata* Gourret and Roeser 1888, which is most certainly a species of *Peritromus* but which Kahl thinks is synonymous with his *P. faurei,* is too long and slender to be confused with *P. tortugensis.* Moreover, the author does not think it identical with Kahl's *P. faurei.* Gourret and Roeser do not show the kind of spines which Kahl shows for his *P. faurei.* At present the author is unable to identify Gourret and Roeser's *Kerona ciliata* as a previously described species of *Peritromus.*

Peritromus gigas is eliminated by its extremely large size, and *P. ovalis* by its shape and the length of its peristome. *Peritromus montanus* is of the same general form and size but has a different type of spine: the spines on the outer or brim portion are probably about the same as in *P. tortugensis,* but those on the central raised crown are more numerous and consist of large, rounded projections, from each of which in turn arises a long, slender spine or cilium. This is entirely different from all other species.

Diagnosis

Peritromus tortugensis n. sp. is very flexible, contractile, only slightly elongated, almost circular in outline, and resembles an old hat in side view, having a raised central crown portion, higher in the rear than in front, and a flattened surrounding bandlike brim; color yellowish; average size approximately 100 × 74 microns; two macronuclei, one in right anterior half, other in left posterior half; body covered with fine cilia both dorsally and ventrally, cilia arranged in fine longitudinal striations, 25 to 30 striations across upper surface; characteristic spines on central raised crown and on the flattened brim; swims in left spirals; turns, without spiraling, to its right side when feeding along over the bottom of the dish.

Trachelocerca dracontoides n. sp.

During the summer of 1930 the author found, in some of the old discarded ameba cultures previously mentioned, an extraordinarily large species of *Trachelocerca.* Again in 1939 it was found in a culture of an unidentified species of yellowish-green algae from the bottom of the moat on the southeast side of Fort Jefferson, after the culture had stood in the laboratory for several days.

Description

Form. This species is characterized by extremely large size, snakelike appearance, and amazing power of contraction and extension. See "Size" below. Cylindrical, wider near the middle of the body, particularly when extended, and gradually narrowed from this point toward the two extremities into a necklike and a tail-like process (fig. 12*A*).

Color. Brownish.

Size. Length (25 measurements) 257 to 2080 microns, average approximately 1289; width (13 measurements) 42 to 100 microns, average approximately 63. When the body is extended to its greatest length, the width is reduced to its narrowest, and conversely, when length is shortest, width is greatest. One individual measured 2080 × 52 microns. The one measuring 257 microns in length was one greatly contracted after it had been jabbed with a steel needle; also the one measuring 100 microns in width was extremely contracted. It is believed that the measurements given here represent about the upper and lower limits of contraction and extension, and are fairly representative.

Striations. Extremely finely and longitudinally striated (fig. 12*B*).

Contractile vacuole. One has been seen, in the middle of the body, somewhat elongated in shape, with rounded ends.

Nucleus. Not seen.

Spiraling. This species usually moves around over the bottom of the dish, and in and out among the débris, in a snakelike manner without swimming and without spiraling. It sometimes swims freely, and may be induced to do so by jerking it up into the water with a steel needle. In such cases it swims jerkily in left spirals.

Conjugation

In August 1935 two individuals were found conjugating, as indicated in figure 13.

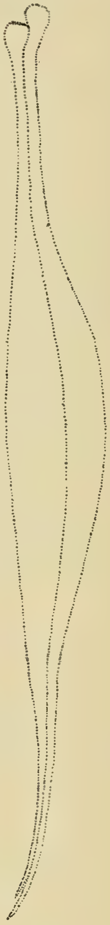

Fig. 13. *Trachelocerca dracontoides* n. sp. Conjugating individuals.

Discussion

Sauerbrey (1928) described a species of *Trachelocerca* under the name *T. fasciolata* which measured up to 1240 microns long by 77 wide. Kahl (1935) reports

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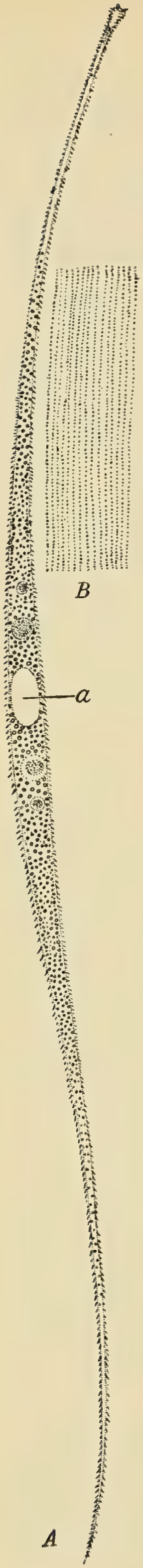

Fig. 12. *Trachelocerca dracontoides* n. sp.

A. Extended view, × 150, showing general size and structure. *a,* contractile vacuole.

B. Section of body, greatly enlarged, showing striations.

finding individuals of this species measuring as much as 3000 microns in length. From the point of view of size alone this species could easily be the one described in this paper. But *T. fasciolata* as described and drawn by Sauerbrey is very wide and flattened for almost its entire length, not cylindrical as is *T. dracontoides,* and has no, or but very slight, tail-like process, the body being rather bluntly pointed posteriorly. *Trachelocerca dracontoides,* on the other hand, has the entire posterior half of the body drawn out into a tail-like process. According to Kahl, also, the tail-like process in *T. fasciolata* is relatively wide, and corresponds in this respect at least to that described by Sauerbrey. But Kahl's drawing of this species is entirely different from that of Sauerbrey. The author believes that the two drawings cannot be reconciled. Sauerbrey's *T. fasciolata* is not *T. dracontoides,* but neither is Kahl's. Nor is Kahl's *T. fasciolata* the same species as Sauerbrey's *T. fasciolata.* In color, however, *T. fasciolata* (according to Kahl) is dark brown, almost the same as *T. dracontoides.*

Trachelocerca phoenicopterus Cohn 1866 is somewhat smaller (maximum length 1500 microns extended, according to Kahl), but in shape is very similar to *T. dracontoides.* The two species have been found in the same culture, however, and when they are seen together a marked difference appears, *T. dracontoides* being larger, more pointed at the posterior extremity, more extended and more snakelike, cylindrical instead of widened and flattened in the posterior half. Also Cohn's drawings of *T. phoenicopterus* show very marked and characteristic transverse striations across the entire body, giving it an extremely serrated appearance. No such striations were found in *T. dracontoides.* Kahl (1927, fig. 20) in his drawings of *T. phoenicopterus* shows a form very similar to that of *T. dracontoides.* Cohn, who first described *T. phoenicopterus* and who supposedly made a characteristic drawing, fails to show this form, but rather has stressed the very prominent serrations, which the author has not seen in *T. dracontoides.*

The only other species (as listed by Kahl, 1935) which is anywhere near large enough to be considered is his *T. coluber,* to which he assigns a length of 1000 microns. The body of this species is very slender, more nearly the shape of *T. dracontoides* than either of the others, but it has a definite short tail-like process, as shown in his drawing, whereas the tail-like process in *T. dracontoides* is the entire posterior half of the body gradually reduced in size all the way to the extreme tip.

Diagnosis

Trachelocerca dracontoides n. sp. is extremely long when extended and may be extremely short when contracted; very slender when extended to its fullest extent, and very characteristically snakelike both in form and in movement; long, slender necklike process, and extremely long, slender posterior tail-like process; body widest centrally, gradually narrowing toward both extremities; cylindrical, not much flattened; very finely and longitudinally striated; one contractile vacuole, elongated, ends rounded, centrally located; average size 1289 × 63 microns. All free swimming has occurred in left spirals.

Stentor (auricula) auriculatus (Gruber 1884) Kahl 1932

Stentor auricula Gruber 1884.

During the summer of 1935 the author found, in an old general culture in a concrete trough back of the laboratory into which various discarded cultures containing considerable quantities of various species of algae, collected from various places, had been dumped, a stentor-like ciliate which differed from any *Stentor* which the author had previously seen.

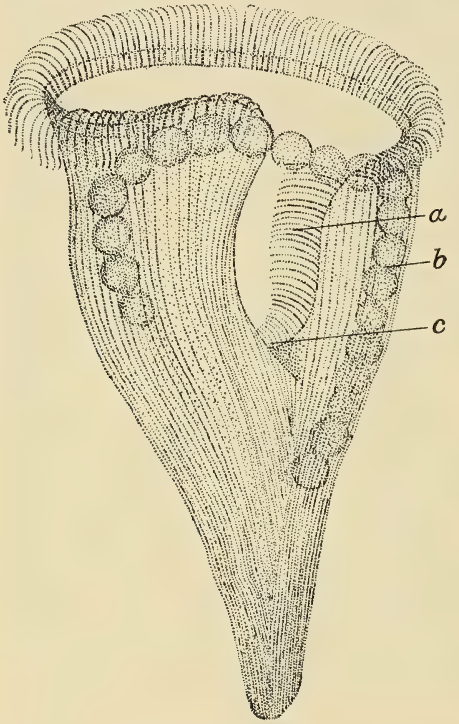

FIG. 14. *Stentor (auricula) auriculatus* (Gruber 1884) Kahl 1932. Ventral view, × 400, showing general form and structure. *a,* pharyngeal cilia; *b,* nucleus; *c,* mouth.

Description

Form. Viewed laterally this species has the characteristic *Stentor* appearance, but viewed ventrally it differs from all other stentors, except *Stentor auricula* Kent (1881–1882), in having a deep cleft in the wall where the spiral-shaped funnel is located in other species (fig. 14). There is some question in the mind of the author whether this should actually be considered a stentor. In general form and reactions, however, it is very much like one.

Size. Length (28 measurements) 87 to 251 microns, average 216; width (14 measurements) 90 to 135 microns, average 119. Only one individual was found measuring as low as 87 microns in length. The width of this particular one was not determined, but the width of the narrowest individual measured (90 microns) was greater than the length of this short individual.

Nucleus. Moniliform, as in *S. coeruleus, S. polymorphus,* and *S. multiformis.* Individual beads mostly spherical, numbering 15 to 20 (fig. 14).

Striations. The body is entirely ciliated, and finely and longitudinally striated (fig. 15).

Peristome. Kahl's species was not much flared out at the peristomial border, but in the one reported here not merely the peristomial border, but the entire anterior half of the body was greatly widened. The immediate peristome border was no wider, or but very little wider, than the body just behind it. In this respect the species observed by the author and the one observed by Kahl were alike. But Kahl in his drawing shows a relatively very narrow body; the one reported here had a relatively wide body. The peristome border is lined with longer cilia.

Contractile vacuole. Not seen.

Spiraling. When swimming freely, this species swims in left spirals which measure approximately 1383 to 1640 microns per spiral, but at times may vary widely. Speed, too, is very variable, but it was not possible to make actual measurements of it.

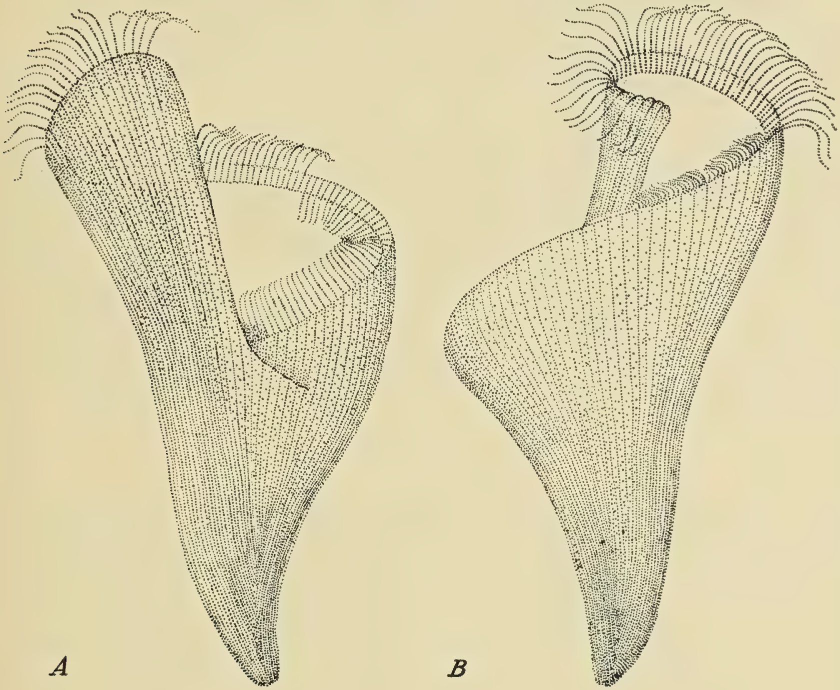

FIG. 15. *Stentor (auricula) auriculatus* (Gruber 1884) Kahl 1932.
A. Ventral view, × 400, showing form variations.
B. Side view, × 400, showing general form variations.

Discussion

Kent (1881–1882) found a stentor-like ciliate, differing from other stentors in that it had a deep cleft in the anterior end of the ventral surface, and had a single spherical nucleus. He gave the name *Stentor auricula* to this species. Gruber (1884) saw a similar animal, but with a moniliform nucleus, which he identified as *Stentor auricula* Kent. Kahl (1932), noting the difference in the nucleus, gave Gruber's species the name *Stentor auriculatus.* Only these two species are known at present to have such a cleft. Neither of these has previously been found in the United States, but Wailes (1928) (according to Kahl, original paper not seen) found the same form in the waters of the Pacific near Vancouver, British Columbia.

Diagnosis

Stentor auriculatus is, in general body form, a typical stentor, but differs in having a deep cleft in the center of the anterior end of the ventral side, where the funnel is located in most stentors; nucleus moniliform, composed of 15 to 20 beads; body entirely ciliated, finely and longitudinally striated; average size approximately 216 × 116 microns; swims in left spirals.

Gruberia (Spirostomum) lanceolatum (Gruber 1884) Kahl 1932

Spirostomum lanceolatum Gruber 1884.
Spirostomum lanceolatum De Morgan 1923–1925.
Gruberia lanceolatum Kahl 1932.

This is a widely distributed species. It was first seen by the author in 1921 at Cold Spring Harbor, in material collected from South Shore salt marsh. In 1923 and 1924 it was found at Cold Spring Harbor in cultures from the large brackish marsh pool back of Jones' boathouse, south end of inner harbor. During the summer of 1925 it was found at Woods Hole in three-day-old culture from a salt pond near the entrance to the Atlantic Ocean from Catama Bay at Edgartown. In 1931 it was found at Tortugas, Florida. During the summer of 1934 it was found in the turtle ponds at the laboratory of the U. S. Bureau of Fisheries, Beaufort, North Carolina.

Description

Form. Elongated, spindle-shaped, narrowest at the two ends, widest in the middle, and very flexible and contractile. Axis of body sometimes straight, often curved extremely or slightly in the shape of a half-moon. The extreme anterior end is about ⅓ to ½ as wide as the body at its widest point, and the anterior half of the body is gradually widened from the end to the center. There is thus a somewhat flattened necklike projection extending back to the mouth, which is about ⅓ to almost ½ the length of the body from the anterior end (fig. 16). The anterior extremity ends in a slight hook or beak extending out over the oral groove. The posterior half of the body is much more pointed, but is not so flat and is not reduced suddenly into a definite tail-like process. It is gradually reduced from the middle of the body to the extreme posterior end.

Color. At least in some specimens, a distinct peach-bloom.

Size. Length (12 measurements) 450 to 1071 microns, average 782; width (9 measurements) 49 to 104 microns, average 75. Width about ⅑ to ⅒ the length.

Mouth and groove. Mouth just anterior to the middle or widest part of the body. The oral groove or peristome extends along the left edge of the anterior half of the body from the extreme anterior end to the mouth, which is slightly to the right of the edge. The groove turns to the right away from the edge at a point just above the mouth. The groove is lined on its right side with a row of longer, more prominent cilia. No undulating membrane has been seen (fig. 16).

Nucleus. More or less moniliform, made up of 10 or more beads, not all the same size. Those seen at Cold Spring Harbor in 1921 had a nucleus made up of spindle-shaped bodies joined end to end and more or less zigzagging lengthwise through the body. Those seen there in 1924 had a nucleus exactly like the one shown in figure 16.

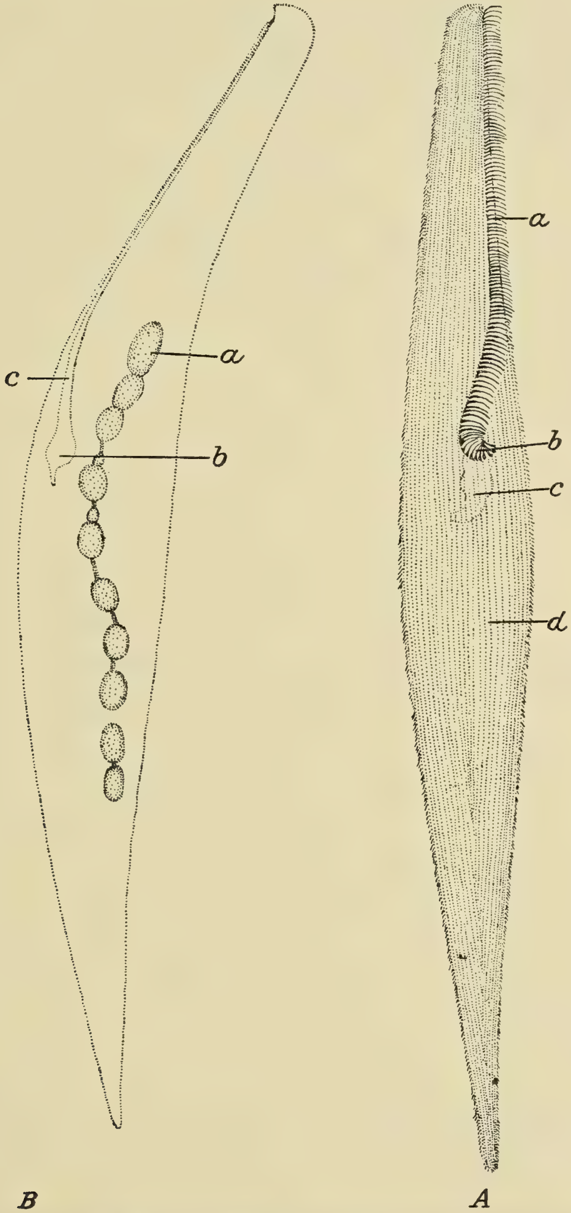

Fig. 16. *Gruberia (Spirostomum) lanceolatum* (Gruber 1884) Kahl 1932.

A. Ventral view, × 200, showing general form and structure. *a,* oral cilia; *b,* mouth; *c,* gullet; *d,* striations.

B. Outline drawing, ventral view, × 200, showing *a,* nucleus; *b,* mouth; *c,* oral groove.

Contractile vacuole. Not seen.

Striations. The body is entirely ciliated, and finely and longitudinally striated, 20 to 25 striations on the upper surface of the body or about 50 to 60 over the entire body, striations about 4 microns wide, granular in appearance (fig. 16; pl. 1, fig. 1).

Spiraling. Very little free swimming has been seen, but most of this has occurred in right spirals measuring about 1100 microns in length. Two individuals at Cold Spring Harbor in 1921 were seen swimming slowly to and from the surface film in left spirals.

Discussion

Kahl (1932) described, under the name *Gruberia uninucleata,* a ciliate having the characteristics of both a *Spirostomum* and a *Blepharisma,* but belonging to neither genus. It had a single oval or elongate nucleus. Gruber (1884) had described a ciliate having similar characteristics but with a moniliform nucleus, under the name *Spirostomum lanceolatum.* De Morgan (1923–1925) found the same species in the tanks at Plymouth, and identified it as *Spirostomum lanceolatum.* But this was clearly not a *Spirostomum,* and Kahl (1932) placed it in the genus *Gruberia* with his *Gruberia uninucleata. Gruberia uninucleata* has a single oval or elongate nucleus, rounded at the ends; *Gruberia lanceolatum* has a moniliform nucleus. The shape of the body is practically the same, but there is some doubt as to the advisability of classifying under the same genus two species with so great a difference in the nucleus. Beltran (1933) described the first and only species from the United States. His species, *G. calkinsi,* has a moniliform nucleus, similar to that of *G. lanceolatum,* and composed of the same number of beads (10), but with its mouth about ⅔ the length of the body from the anterior end, instead of ⅓ as in *G. lanceolatum.* Moreover, the necklike extension in *G. calkinsi* is practically the same width its entire length except just in front of the mouth; that of *G. lanceolatum* is gradually narrower from the mouth to the extreme anterior end. In *G. lanceolatum* the posterior half of the body is cylindrical, not flattened, and is also gradually reduced from the middle of the body to the extreme posterior end. The posterior end of *G. calkinsi* is much shorter, not equal to half the body, and comes more quickly to a point. Finally, the striations in *G. lanceolatum* are very fine; those in *G. calkinsi* (according to Beltran's drawing) are coarser (wider apart) and separated by a relatively wide band of fine granules.

Diagnosis

Gruberia lanceolatum is very much elongated, spindle-shaped; wider in the middle of the body, smaller at the two ends, flattened anteriorly and ending in a slight beaklike projection, more pointed posteriorly, flexible, contractile; body sometimes straight, but probably more often curved; mouth about ⅓ to ½ the body length from anterior end, just anterior to widest part of body; oral groove down left edge of necklike extension; color peach-bloom; body finely and longitudinally striated, 20 to 25 striations on upper surface; average size approximately

782 × 75 microns; swims in right spirals normally, but may also swim in left spirals.

Strombidium alveolare n. sp.

This species was found in 1935 on fresh floating material at the dock at Fort Jefferson, at high tide.

Description

Form. Somewhat elongated, bluntly rounded at each end, constricted just anterior to the middle of the body; peristome extends down almost to this constriction; body flattened dorsoventrally (fig. 17).

Color. Clear or colorless.

Size. Length (12 measurements) 54 to 75 microns, average 59; width (5 measurements) 31 to 46 microns, average 37.

Nucleus. Not seen.

Contractile vacuole. Not seen.

Vacuolation. This species is characterized by its extreme alveolar or vacuolated appearance. The body seems one mass of globules.

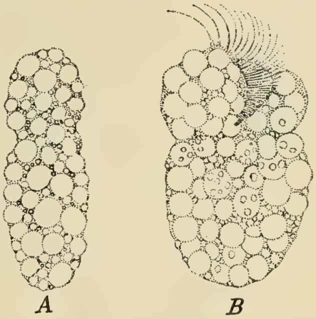

FIG. 17. *Strombidium alveolare* n. sp.

A. Edge view, × 500, showing general form, size, and vacuolated structure.

B. Ventral view, × 500, showing characteristic form and structure.

Discussion

This is being described as a new species on the basis of the extreme vacuolated condition of the protoplasm, its constriction near the anterior third of the body, and its general shape—elongated, flattened, and rounded at the ends (fig. 17). No other species known to the author shows this structure. The shape is somewhat like that of *Strombidium cinctum* Kahl (1932), which measures 40 to 50 microns, and is somewhat flattened, obovoid or oblong. But *S. cinctum* is too long and slender, and is not vacuolated. The body form also somewhat resembles that of *S. armatum* Burger (1908) (according to Kahl [1932]; Burger's original article not seen), which measures 50 to 60 microns. The shape is more like that of *S. purpureum,* which measures 40 to 50 microns, but this species is purplish in color and is not vacuolated. *Strombidium minutum* Wulff (1919) (according to Kahl [1932]; Wulff's original article not seen) seems to be somewhat vacuolated in structure, but it is too conical in shape and the body is too small. No body form, out of forty-nine species listed by Kahl, seems exactly to fit this species, and no species has been described with a similar structure.

Diagnosis

Strombidium alveolare n. sp. is somewhat elongated, rounded at the ends, rather sharply constricted just anterior to the middle of the body, somewhat flattened dorsoventrally; measures approximately 59 × 37 microns; characterized chiefly by its extremely vacuolated structure and greatly flattened body.

Epiclintes caudatus n. sp.

This species was found at Tortugas during the summers of 1931 and 1939. There is no record as to the exact source of the material from which it came.

Description

Form. A very long, slender hypotrich, widest centrally, dorsoventrally flattened, extremely extensile and contractile, extending its body sometimes to great lengths. A relatively long, slender necklike process extends forward from the widened middle part of the body and widens out again at the anterior end around the peristome, almost but not quite to the width of the middle of the body. The posterior half of the body is reduced rapidly just back of the widened central portion to an extremely long, narrow tail-like process, of about the same width its entire length, only slightly enlarged at the tip (fig. 18).

Color. Yellowish.

Size. Length (12 measurements) 260 to 440 microns, average 354; width (6 measurements) 22 to 44 microns, average 28.6. Width about ⅒ to 1⁄14 the length.

Spines. Short, blunt spines of a peculiar type, almost transparent, line the sides of the body, beginning at a point about midway of the peristome and extending to the extreme tip of the tail-like structure. They are located at regular intervals along the sides of the body, about 65 to a side, and were the first structures to attract attention in this species. Later the spines were found to be a generic characteristic (fig. 18).

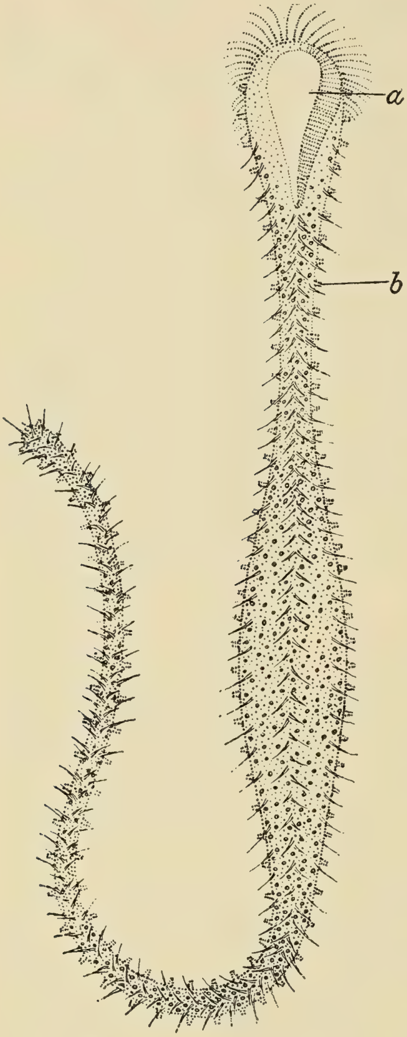

FIG. 18. *Epiclintes caudatus* n. sp. Ventral view, × 500, showing characteristic form and structure. *a*, peristome; *b*, one of body spines which characterize the group.

Cirri. Four longitudinal rows of cirri on the ventral surface: 1 row near each edge (marginal cirri) and 2 near the mid-line (ventral cirri) (fig. 18).

Spiraling. In moving freely, individuals swim in left spirals, but when running around over the bottom of the dish, or on the under side of the surface film, they move without spiraling, in circles, sometimes to their right, sometimes to their left.

Discussion

This ciliate is a hypotrich, and is identified as a member of the genus *Epiclintes* because of the body shape, lateral spines, shape of peristome, and ventral cirri. It is very similar to Butschli's (1889) drawing of his *Epiclintes ambiguus (Trichoda ambigua* O. F. Müller 1786). The necklike process in *E. ambiguus,* however, is very short and wide; in *E. caudatus,* very long and slender. The tail-like process, although of about the same proportionate length in each, is much wider and not so tail-like in *E. ambiguus;* much more slender, very definitely tail-like in *E. caudatus.* The short spines along the edge of the body, which characterize *E. caudatus,* also characterize *E. ambiguus* (Butschli), but in the latter they are found over the entire upper surface, as indicated in his side-view drawing; in *E. caudatus* they have been seen only along the edges of the body, one row down the left edge and one down the right. It is quite possible, however, that they are present over the entire upper surface and have not been seen, since no individual has been seen except in surface view. Moreover, according to Butschli, *E. ambiguus* has 6 rows of cirri running longitudinally over the ventral surface of the body, 2 marginal and 4 ventral. Only 4 rows have been seen on *E. caudatus,* 2 marginal and 2 ventral. Kahl (1932) in his drawing of *E. ambiguus* shows 10 rows of cirri on the ventral surface, but instead of running longitudinally, as in Butschli, they are shown running across the body, at an angle to the animal's right, beginning at the posterior end and going toward the anterior end. By the time the anterior end is reached the last row is practically perpendicular to the long axis. This gives an entirely different idea of this species, and since it is supposed to be redrawn from Butschli, the drawing is incorrect. Both *E. ambiguus* and *E. caudatus* are yellowish in color, both are wider centrally, and both are very contractile, but *E. caudatus* has not so definite an ear-like peristome.

Diagnosis

Epiclintes caudatus n. sp. is an extremely long, narrow ciliate, with extreme power of extension and contraction, a very long, distinct tail-like process, a distinct necklike process, and a widened anterior extremity; middle of body is widest part, but anterior peristomial area is only slightly less wide. Lateral spines; 2 lateral (marginal) and 2 ventral rows of cirri running lengthwise of the body; peristome has less resemblance to an ear than in *E. ambiguus;* average size about 354 × 28.6 microns; swims in left spirals.

Uncinata gigantea n. gen. and sp.

This species has been seen twice at Tortugas, the last time during the summer of 1939. Only a few individuals were seen the first time, and only two the second time. It is thought to have come from Long Key, but this is not certain.

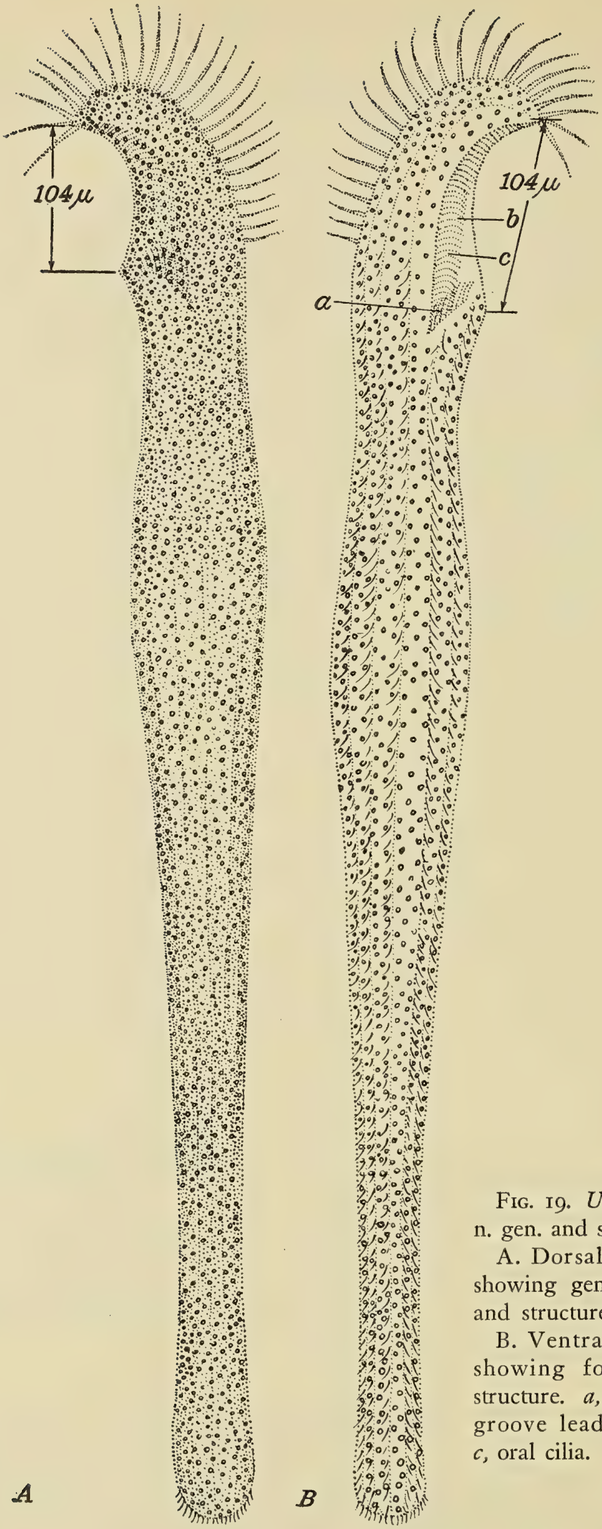

Fig. 19. *Uncinata gigantea* n. gen. and sp.

A. Dorsal view, × 300, showing general size, form, and structure.

B. Ventral view, × 300, showing form, size, and structure. *a,* mouth; *b,* oral groove leading to mouth; *c,* oral cilia.

Description

Form. Extremely long, even when contracted, but can be extended greatly; very soft and flexible. Very prominent characteristic beaklike projection at anterior end, which curves to the animal's left. Body very definitely and sharply expanded at mouth region, narrowed posterior to this point for a short distance, then widened again. This latter is the widest part, and is about ⅓ the length of the body from the anterior end. Posterior to this point, the animal is narrowed to about ⅔ the width at the widest point. This narrowed region extends all the way to the posterior end, but is not a definite tail-like structure as in *Epiclintes caudatus.* Extreme posterior end rounded, slightly enlarged (fig. 19).

Color. Yellowish, about the same as *Epiclintes caudatus.*

Size. Length (20 measurements) 264 to 1100 microns, average 618; width (12 measurements) 25 to 75 microns, average 47. As in *Trachelocerca,* the width decreases as the animal extends, and increases as it contracts. A variation, then, of from 264 × 75 to 1100 × 25 microns is approximately correct, but since no measurements of both length and width have been made upon the same individual in both the extended and the contracted condition, the exact dimensions in such cases cannot be stated.

Ventral cirri. Five rows of ventral cirri or styles running lengthwise of the body. Three of these run from the extreme posterior end to the beak at the extreme anterior end; the other two stop at the mouth (fig. 19). No caudal cirri, transverse, frontal, or other special cirri have been seen.

Beaklike anterior end. The anterior end of the body ends in a beaklike projection (uncinus) which extends several microns beyond the left body wall (fig. 19). No such beak is known in any other ciliate described to date. It is this prominent beaklike extension of the anterior end of the body which has given rise to the generic name, *Uncinata.* So far as it has been possible to determine, this has never been used as a generic name before, and therefore is available.

Oral groove and mouth. A groove extends in a smooth curve from the extreme tip of the beak back to the mouth, located at the first enlargement of the body, about 104 microns from the beak (fig. 19). Mouth almost in the midventral line.

Spiraling. This species has been seen swimming only once. This was in left spirals. Spiraling was not observed in 1939.

Discussion

The shape of the body and its extreme length, particularly when extended, and the enormous power to extend and contract are very striking. During the summer of 1939, however, a much smaller species, about one-third the size of the one above, was seen, and for a short time was mistaken for the giant species. This smaller species, after it was discovered to be different, was not found in large numbers, and every time an attempt was made to transfer a few of the individuals to a slide for study under the compound microscope they went to pieces. Of six to eight attempts, not one was successful. For this reason their study was not completed.

Diagnosis

Uncinata gigantea n. gen. and sp. is an extremely long ciliate with two expansions in its body—one at the mouth, the other a short distance back of the mouth—and with a very prominent beaklike projection to the left at the anterior end, characteristic of the genus; body highly extensile and contractile; mouth midway between right and left sides, about 104 microns from point of beak; color yellowish; 5 rows of cirri on ventral surface, 2 marginal and 3 ventral; posterior half to two-thirds reduced somewhat in size but not constituting a definite tail-like process; average size approximately 618 × 47 microns.

Holosticha (Oxytricha) rubra (Ehrbg. 1838) Kent 1881–1882

Oxytricha rubra Ehrbg. 1838.
Oxytricha rubra Duj. 1841.
Oxytricha rubra C. & L. 1858.
Holosticha rubra Kent 1881–1882.
Holosticha flaborubra Entz 1884.
Holosticha (Keronopsis) rubra Kahl 1932.

This very interesting hypotrich ciliate was first seen at Tortugas during the summer of 1931. During the summer of 1934 it was found at Beaufort, North Carolina (laboratory of U. S. Bureau of Fisheries), in culture one to two weeks old from the channel, where it had been collected from along the wall at low tide. It was found again at Tortugas during the summer of 1935 in cultures from Bush Key and around Loggerhead Key. In 1939 it was found in large numbers in cultures of algae from near the tip of Long Key, at low tide.

Description

Form. Elongated, widest centrally, narrowed throughout the posterior fourth or fifth into a projection about ½ the width of the body at its widest point. Both posterior and anterior ends rounded; anterior end narrowed and pointed somewhat to the animal's left as a sort of peristomial lip. Peristome itself relatively large and lined on its right side with one rather long and one shorter row of slender styles or cirri. Body very contractile and extremely soft and flexible (figs. 20, 21).

Color. A reddish brown which, when the animals are thinned out under a cover glass and studied under the 4-mm. objective and the 10 × ocular, is found to have a yellowish cast.

Size. Length (76 measurements) 172 to 264 microns, average 206; width (55 measurements) 36 to 66 microns, average 49. Kahl (1932) reports length varying from 200 to 300 microns. Kent (1881–1882) gives length as about 178 microns.

Dorsal spots. On the upper or dorsal surface, as seen under the binocular microscope, are 5 to 6 longitudinal rows of prominent, deep reddish-brown spots, 9 to 11.5 microns apart, which appear as small rings under the 16-mm. objective compound microscope. When examined under the 4-mm. objective and the 10 × ocular they are found to be not solid rings, but rings of small granules (fig. 20).

Granules. The entire body is filled with small reddish granules, smaller than those composing the rings. A massing of these granules in the anterior tip of the body gives somewhat the appearance of a pigment spot.

Ventral rows of granules. On the ventral side are two very prominent peculiar rows of larger granules, one on either side of the mid-ventral line. These rows look somewhat at times like rows of the letter X placed end to end except that the right side of the X is heavier, more prominent. In or near the mid-ventral line is a third row of granules similar to the other two, but composed of fewer granules, and with the X arrangement less prominent; one side of the X seems to have been left out (pl. 1, fig. 2).

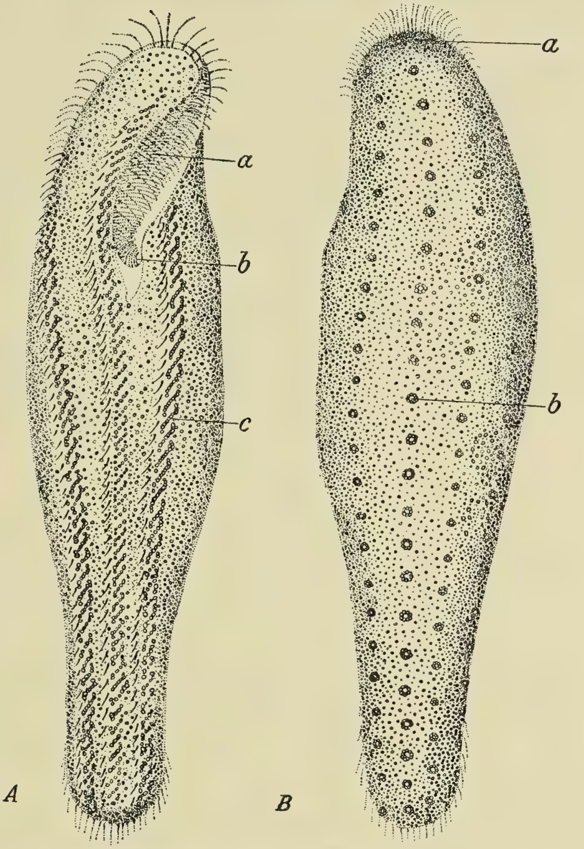

Fig. 20. *Holosticha rubra* (Ehrbg. 1838) Kent 1881–1882.

A. Ventral view, × 500, showing general form, size, etc. *a,* pharynx; *b,* mouth; *c,* rows of characteristic granules.

B. Dorsal view, × 500, showing form, rows of dorsal granules, etc. *a,* dense mass of reddish granules; *b,* small circles of larger granules arranged in longitudinal rows.

Ventral cirri. Alongside each row of ventral granules is a row of relatively small, slender cirri, 3 rows all together. Marginal cirri may be present but have not been seen (fig. 20).

Spiraling. This species when swimming freely swims in left spirals, but free swimming does not often occur and is difficult to induce. Usually spiraling is induced by jerking the animals to the surface with a steel needle; they then move in spirals as they gradually sink to the bottom of the dish.

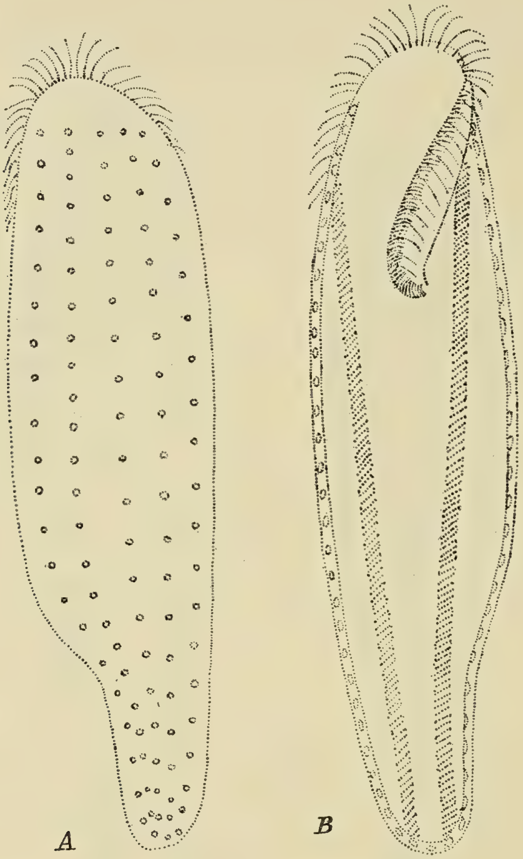

Fig. 21. *Holosticha rubra* (Ehrbg. 1838) Kent 1881–1882.
A. Dorsal, and
B. Ventral views, × app. 500, showing form variation.

Discussion

Holosticha rubra has, so far as it has been possible to determine, never been reported from this country before, but from these investigations it seems fairly plentiful and rather widely distributed. It has not previously been properly shown; neither the rows of ventral granules nor the rings of dorsal granules have been correctly drawn. Kahl (1932) in his two hurried sketches gives a rough idea of the dorsal surface, but no one could recognize the animal from his drawing. His representation of the ventral rows gives no idea of their real appearance. Butschli (1889) shows the dorsal rows of granular circles as solid spheres or vacuoles, which is incorrect. He gives no ventral view.

Diagnosis

Holosticha rubra is elongate, slender, widest centrally, anterior fourth narrowed; peristomial lip projects out slightly to animal's left; peristome fairly large and lined on the right side with apparently two sets of styles or cirri, one longer than the other; posterior third reduced to about ½ body width at widest point; both ends rounded; 3 ventral rows of granules running lengthwise and arranged much like a series of letter X's placed end to end; 5 to 6 dorsal rows of relatively large circles of granules, about 10 granules to the circle, running lengthwise entire length of body. Color reddish, with a yellowish cast; dense mass of reddish granules, smaller than those in the circles and ventral rows, located in tip of the anterior end, forming a sort of eye spot; this spot was not general, however, nor very prominent; average size approximately 206 × 49 microns; free swimming rarely seen, difficult to induce, has occurred only in left spirals.

Uronychia heinrothi Buddenbrock 1920

This species was seen first in 1930, in a discarded ameba culture collected by Dr. D. L. Hopkins, but it has been seen more or less regularly since then in cultures from various localities in the Tortugas region.

Description

Form. As found at Tortugas, normally like *Uronychia transfuga,* but much larger. Buddenbrock (1920) shows a distinct uncinate process on the posterior extremity, but not so large as that found by Taylor (1928) on his *U. uncinata.* The author has seen no such process on those at Tortugas.

Color. Pinkish.

Size. This species became interesting at first because of its large size. As found at Tortugas, the length (37 measurements) is 139 to 264 microns, average 196; width (37 measurements), 83 to 132 microns, average 114.

Nucleus. Very much elongated and composed of about 8 oval bodies joined together much like a string of beads (moniliform structure) except that the composing beads are more or less fused into one another (fig. 24*A*).

Ventral membranes. The second thing that attracted attention to this species was the ventral membranes attached around the edges of the peristome. There are 5 to 6 of these, extremely large and apparently very powerful, not mere undulating membranes.

The largest of these membranes is attached on the right border of the peristome and extends from near the upper edge of the right border down the entire right border and across the posterior border (fig. 22, *c*). Another large membrane is attached to the entire anterior border (fig. 22, *b*). On the left border are two smaller membranes (fig. 22, *a, d*). Below these is a smaller membrane (not shown in the drawing) divided into four parts. There is one other very large, powerful membrane attached outside the border of the peristome along, or near to, the right-hand border of the body (fig. 22, *e*). This membrane, shown here extending beyond the body wall, beats in toward and over the peristome with great force. All the other membranes also beat in toward the center of the peristome and thus serve to create a strong current to bring food into the mouth. But while they were being studied the author became convinced that they also served as swimming structures.

These membranes have not previously been very satisfactorily described or shown in drawings. Buddenbrock (1920) shows in outline the five membranes which surround the peristome, and shows them extended beyond the body wall. He fails to show the large one attached near the edge of the right body wall. Pierantoni (1909) shows two very long flaplike membranes on the right and left border of the peristome in *Uronychia transfuga* (fig. 23). He shows similar structures for his *U. magna.* Taylor (1928) shows on his *U. uncinata* two extremely large, wide flaps (membranes) somewhat similar to those shown for *U. transfuga* by Pierantoni. Kahl (1932) in his description of the genus describes six or more peristomial membranes, but they cannot be made out from his drawings.

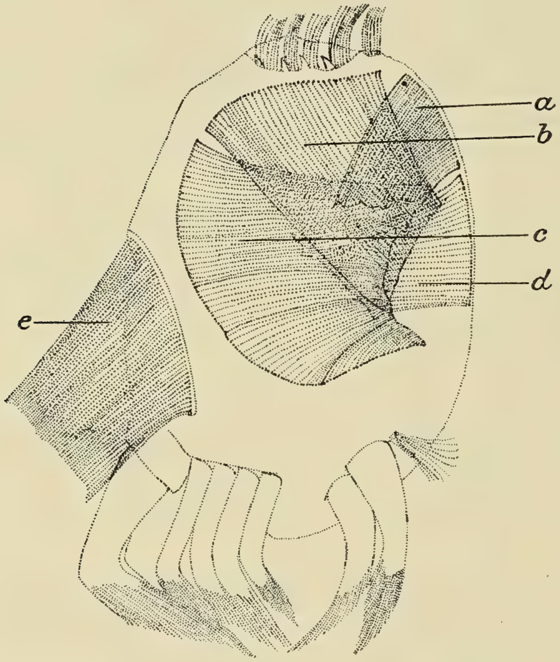

Fig. 22. *Uronychia heinrothi* Buddenbrock 1920. Ventral view, × 400, showing peristomial membranes. *a,* small membrane attached to upper left border of peristome; *b,* large membrane attached across anterior border of peristome; *c,* very large membrane attached to right side and posterior border of peristome; *d,* small membrane attached to lower part of left border of peristome; *e,* large membrane attached to lower right section of body outside of the peristomial zone.

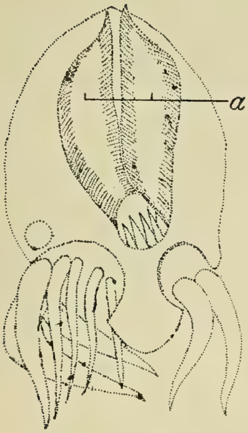

FIG. 23. *Uronychia (Trichoda) transfuga* (O. F. Müller 1786). Redrawn from Pierantoni to show ventral undulating membranes. *a*, peristomial membranes.

Posterior cirri. While the peristomial membranes were being studied it was discovered that the extremely large and powerful posterior cirri, known to be swimming organelles, did not derive their power from contracting as single structures. When they contract, their outer third or half becomes fimbriated or frayed, the composing cilia separating and spreading out fanwise to become a membrane or sheet of cilia joined side by side and held together by a film of water (fig. 24*A*). These cilia, attached at the base as a single structure, beat together as a unit and thus develop extraordinary power. Such a structure is comparable to the blade of an oar in contrast with a pole without a blade, or to an open fan as contrasted with a closed fan. Apparently the cirri can be broken up into their composing cilia at any time, but chiefly in preparation for jumping and swimming movements. When the animals come to rest the cilia assume their normal position in the cirri. It seems that this separation of the composing cilia either is automatic as the animal starts to move, or is under the control of the organism and is resorted to deliberately (using the term "deliberately" reservedly) in all free movement.

Previous investigators have reported this fimbriated condition and have shown it in their drawings, both for *Uronychia* and for other Hypotricha. None, however, seems to have associated it in a definite way with locomotion. Claparède and Lachmann (1858) showed fimbriation in the outer ends of the posterior cirri in their *Campylopus paradoxus (Uronychia).* Kent (1880–1882) shows fimbriation in the outer ends of the posterior cirri of *Uronychia transfuga.* Taylor (1928) reports fimbriation in his *U. uncinata,* but thinks it associated in some way with the process of cell division. He seemed to find it at the time of, or just preceding, division. This has not appeared to be the case in the species under discussion here.

Dorsally attached posterior cirri. During the summer of 1939, two previously unsuspected and extremely powerful cirri were discovered attached to the dorsal body surface of this species, near the mid-line in the posterior third. These cirri in normal resting condition project posteriorly beyond the body at the right side, where they bend sharply to the left and extend over to meet the cirri attached on the left side (fig. 24*B, a*). In this position they lie alongside one other large cirrus from the right edge of the body, which is also attached dorsally, but in a small pocket slightly beneath the other two (fig. 24*B, b*). This ordinarily gives the appearance of three large bent cirri, of about the same size, attached in a row across the right side of the body. All three dorsal cirri have been seen by previous investigators and are not being reported here as new, but their action and the fact that they are attached to the dorsal surface is not believed to be

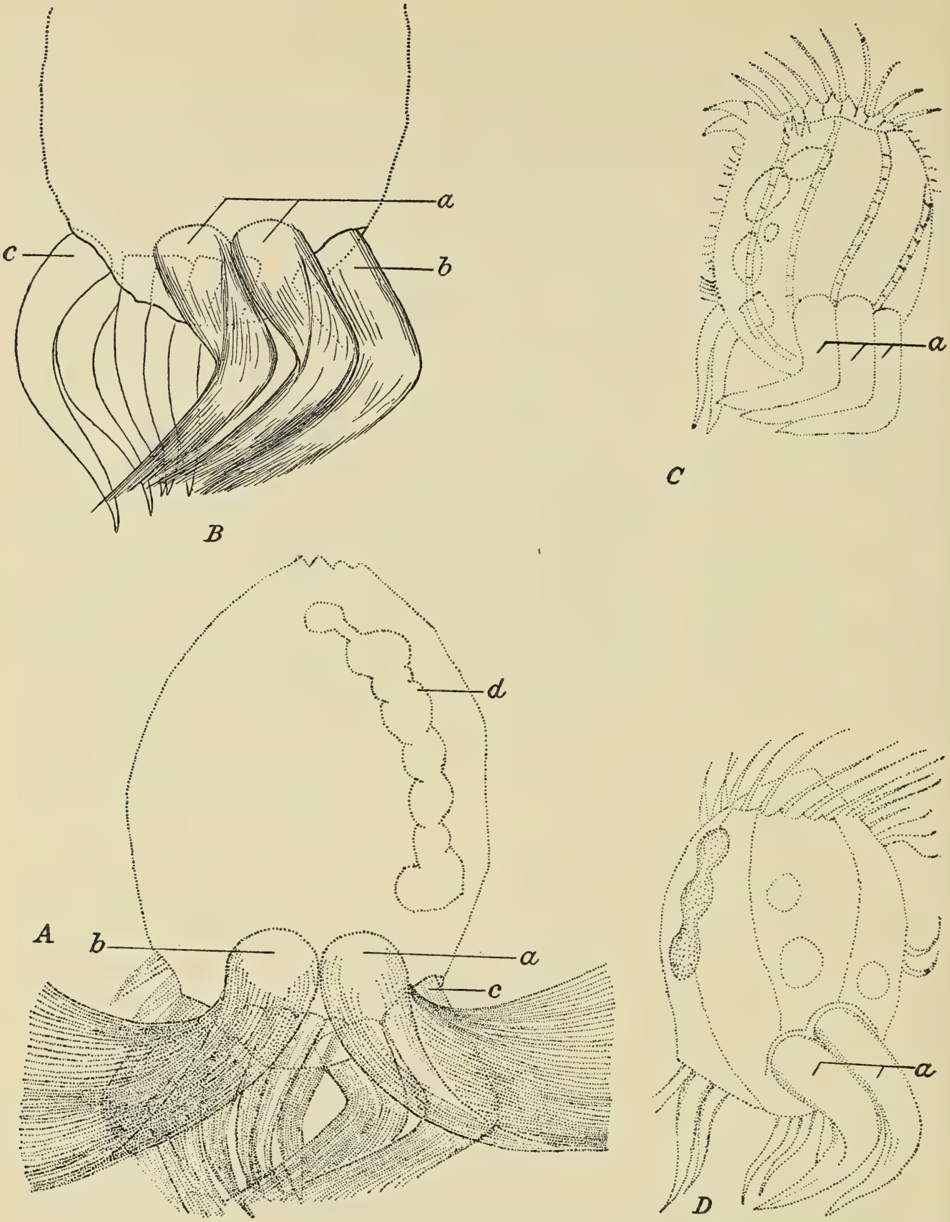

Fig. 24

A. *Uronychia heinrothi* Buddenbrock 1920. Dorsal view, × 400, outline to show nucleus and posterior cirri. *a,* dorsal cirrus attached to right of mid-line and showing fimbriation; *b,* second dorsal cirrus to left of mid-line, also fimbriated; *c,* third dorsal cirrus attached in pocket near right edge and slightly below first two; *d,* nucleus, showing general structure.

B. *Uronychia heinrothi* Buddenbrock 1920. Dorsal view, × 400, showing the three dorsal cirri lying side by side. *a,* the two large cirri attached in the mid-dorsal region of the posterior end; *b,* the right edge cirrus; *c,* outer, ventral, curved cirrus.

C. *Uronychia (Trichoda) transfuga* (O. F. Müller 1786). Redrawn from Kahl to show the three large dorsal cirri (*a*).

D. *Uronychia (Trichoda) transfuga* (O. F. Müller 1786). Redrawn from Pierantoni to show the two centrally located dorsal cirri (*a*).

generally known. Kahl (1932) shows three large bent dorsal cirri in *U. transfuga,* all at about the same level, and at the right side of the posterior end (fig. 24*C*). Pierantoni (1909) shows only the two large central, dorsally attached, posterior cirri, in the same species, and the arrangement is slightly different (fig. 24*D*), the right cirrus being attached slightly higher on the body. Buddenbrock (1920) shows three large dorsally attached cirri in *U. heinrothi,* all in a row, with no differentiation into marginal and central cirri.

Moreover, it is probably not generally known that *Uronychia* has both dorsal and ventral cirri. The Hypotricha, to which these animals belong, are usually considered as having cilia only on the ventral surface of the body, and those cilia modified into special structures called cirri or styles. Here, however, are hypotrichs with modified cilia on both the dorsal and ventral surfaces.

The two central, dorsally attached cirri (fig. 24*B, a*) are not always bent to the animal's left, nor are they always lying alongside each other. In movement, they too spread out their cilia fanwise, and may at times point in opposite directions, one to the right, the other to the left (fig. 24*A, a, b*). The composing cilia form a very wide sheet, similar to that of the other posterior cirri, and the two cirri then beat forward directly over the dorsal surface of the body. In this way they can develop enormous power. The other posterior cirri also beat toward the anterior end, but only up to the posterior end of the body. The stroke of the two central dorsal cirri is, therefore, much longer and should be more powerful.

Spiraling. These animals swim backward; none have ever been seen swimming forward. They pull themselves along with the powerful forward-directed beat of all their fimbriated posterior cirri. This swimming is always in right spirals, as in all backward-swimming *Paramecium, Frontonia,* etc. (Bullington, 1930, 1939).

In 45 measurements of length, these spirals have averaged 1495 microns, varying from 1037 to 2075. Of only 2 measurements of width of spiral, one giving 132 microns, the other 176, the average is 154 microns. The average speed has been about 7257 microns per second, varying from 6385 to 8300.

Discussion

Uronychia heinrothi, as described by Buddenbrock (1920), measured 70 to 250 microns in length as compared with a length of from 129 to 264 microns in those found at Tortugas. The nucleus in his form was made up of 5 to 10 partially fused beads, as compared with 8 in the one at Tortugas (there are only 3 in *U. transfuga).* The peristome in his form was lined with 5 membranes, as compared with 4 (5 counting the small one on the lower left-hand border, near the mouth) on the peristome of the one from Tortugas. Buddenbrock shows no membrane comparable to the one located to the right of the right-hand border of the peristome seen in the Tortugas individuals. These membranes differ from those shown for other species. Taylor (1928) shows for his *U. uncinata* only two wide flaplike membranes running lengthwise through the body and attached one on either side of the peristome.

Uronychia transfuga, according to Calkins, measures approximately 40 × 25 microns; its length, according to Kahl (1932), varies from 50 to 150 microns; according to Kent (1881–1882), is about 86 microns; and according to Pierantoni (1909), varies from 70 to 80 microns. It is therefore considerably smaller than *U. heinrothi.*

Diagnosis

Uronychia heinrothi, as described here, is an extremely large species of *Uronychia,* much like *U. transfuga* in general form, but much larger; average size approximately 196 × 114 microns; nucleus composed of 5 to 10 or more fused beadlike parts (the author found 8; Buddenbrock found from 5 to 10); 5 to 6 large, powerful ventral membranes surround the peristome, all but one of which are attached to its border. Posterior cirri 10 in number (9 shown by Buddenbrock): 2 curved, ventral, located on the left posterior edge; 4 smaller, more slender, straight or curved, ventral, located in the center a short distance anterior to the posterior extremity; 2 large, bent (located on the right side), one ventral, the other dorsal, the dorsal one located in a pocket at the right edge of the body, the ventral slightly to the left; and 2 bent, dorsal, located in the mid-line on the upper surface of the body, slightly in advance of the point opposite the 4 ventral cirri. Swims backward by means of the posterior cirri, always in right spirals measuring about 1500 microns in length and at an average speed of approximately 7000 microns per second.

Gastrocirrhus stentoreus n. sp.

During the summer of 1939 a ciliate was found which was peculiar in that it combined the characters of both heterotrich and hypotrich. No record was made as to the exact source of the material in which it was found, but it is believed to have come from Long Key.

Description

Form. These ciliates attracted attention at once because of their peculiar shape, like that of a barrel with the upper third cut off and the lower end rounded or bluntly pointed. If a wide groove is cut in one side extending down almost ⅔ the length of what is left of the barrel, and sharply curved to the right at the bottom, and if the edges of this groove are mashed in somewhat toward the opposite wall, we shall have an almost exact duplication of the form of this animal. The end from which the upper third was cut is the upper or oral end, and is surrounded by a wreath of long cilia, as in the Heterotricha. The somewhat flattened side with the wide groove is the ventral side (fig. 25). This animal resembles *Stentor auriculatus* probably more than it does any other ciliate, but it is less long drawn out, much more blunt, and wider in proportion to length at the upper end. It resembles a species of *Condylostoma* in the relatively wide peristome leading to the mouth, and in the undulating membrane bordering the left side. It resembles *Bursaria* in the funnel-like pharyngeal extension of the peristome, except that this extension turns to the right instead of to

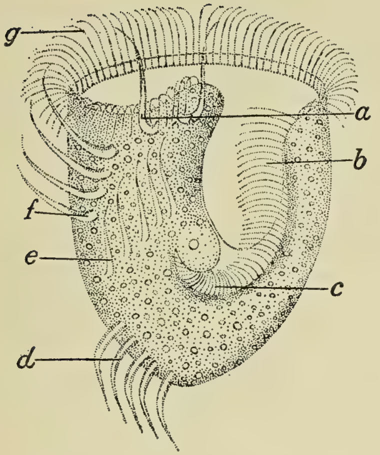

Fig. 25. *Gastrocirrhus stentoreus* n. sp. Ventral view, × 500, showing general form and structure. *a,* two long anterior styles or cirri; *b,* oral membrane or oral cilia; *c,* pharynx; *d,* 5 caudal bent cirri; *e,* 5 smaller ventral cirri; *f,* a group of 4 anterior border cirri; *g,* oral cilia.

the left as in *Bursaria.* But it is also like a hypotrich in that it has cirri grouped over the ventral side.

Color. Not noted.

Size. Only a few measurements have been made. Length approximately 104 microns; width 71 to 81 microns. Width about ¾ the length.

Contractile vacuole. Not seen.

Nucleus. Not seen.

Endoplasm. Granular, rather dark. No striations of any sort and no general body cilia have been seen.

Cirri. The cirri, 16 in number, seem to be limited to the ventral surface and are arranged in four groups: 5 prominent caudal cirri, at the animal's right posterior extremity, where they project beyond the body and bend to the left; 5 ventral cirri, just anterior to the middle of the right half of the body, arranged in a row running diagonally from the lower right side toward the middle of the peristome and near the mouth at the end of the funnel; 4 marginal cirri, on the animal's right side, above the ventral cirri, running up the body in a longitudinal direction or at a slight angle to the left; 2 somewhat isolated anterior or oral cirri, almost on the anterior edge of the ventral body wall (fig. 25).

Movement. These ciliates move by jumping or springing, and also by swimming at high speed. No spiraling has been detected. In free swimming the animal probably moves in spirals, but because of its jumpy movements and great speed, very careful study is required to see this. Such study is still to be made.

Discussion

The generic name *Gastrocirrhus* was created by Lepsi (1928) for his new and interesting ciliate having the characteristics both of a heterotrich and of a hypotrich, which he found on the coast of Palestine near Beirut, and to which he gave the name *Gastrocirrhus intermedius.* His species was of about the same shape as the one described here as *Gastrocirrhus stentoreus.*

The ventral cirri on Lepsi's species are smaller and shorter (as shown in his drawing) than those on *G. stentoreus,* and are not grouped in the same manner. He shows three groups of 3 each down the right border. The author has found two groups down the right border, namely, the 4 marginal cirri and the 5 caudal cirri described above. In addition, *G. stentoreus* has 5 ventral cirri and 2 oral cirri. Lepsi shows neither of the latter groups, but shows instead a group of 7 more slender cirri or styles extending around the posterior end and up part way on the animal's left side. No such cirri or styles have been seen on *G. stentoreus.*

Moreover, *G. stentoreus,* measuring 104 × 71–81 microns, is larger than *G. intermedius,* which measured 68 microns in greatest diameter.

Kahl (1935) was unable to classify Lepsi's species and was inclined to think it a fragment of some oxytrichan.

Diagnosis

Gastrocirrhus stentoreus n. sp. has a form like a barrel with the upper third cut off and the lower end rounded or bluntly pointed; a relatively wide groove in the ventral side, extending posteriorly about ⅔ the length of the body; edges of groove curved in toward the opposite wall; 16 cirri on ventral side, in four groups: 5 caudal, 5 ventral, 4 marginal, and 2 oral. Approximate size, 104 × 71–81 microns; movement, usually a sort of jumping or springing, but also occurs by swimming at high speed.

CONCLUSION

The species here discussed and described constitute only a few of those which have been seen at Tortugas at one time or another, but these are all the author feels justified in discussing, at the present time, with the information at hand. He hopes at some later date to be able to make available a more complete list.

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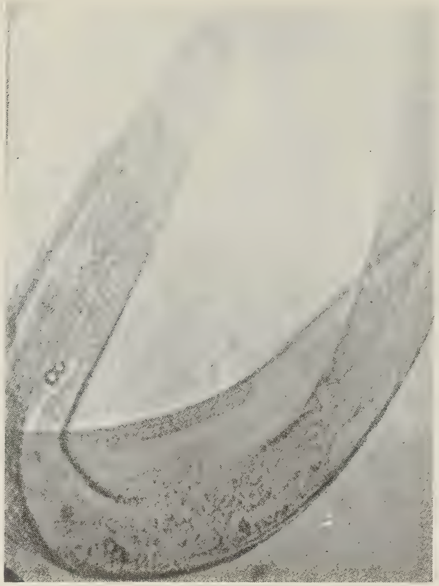

FIG. 1. *Gruberia (Spirostomum) lanceolatum* (Gruber 1884) Kahl 1932. Microphotograph, × 200, showing striations, general shape, etc.

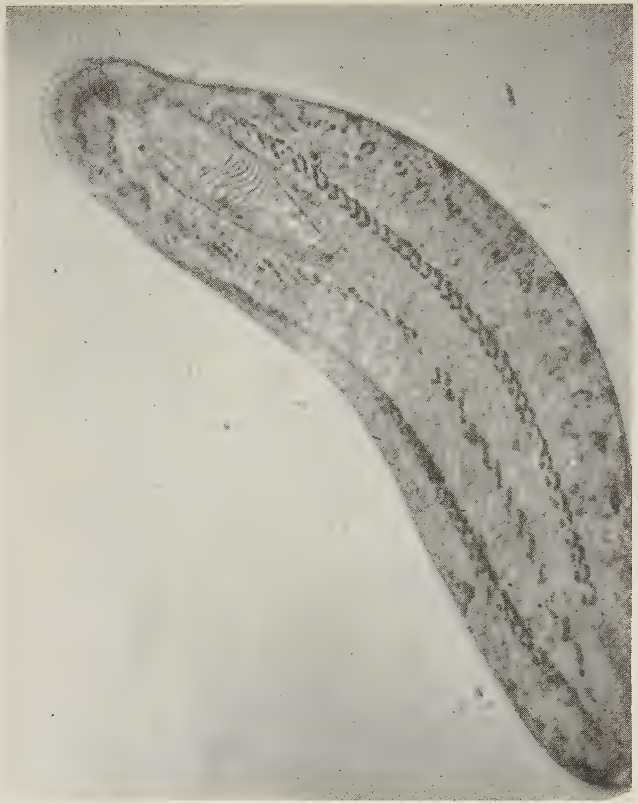

FIG. 2. *Holosticha (Oxytricha) rubra* (Ehrbg. 1838) Kent 1881–1882. Microphotograph, × app. 400, showing general structure, oral and pharyngeal cilia, rows of ventral granules, etc.

PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

XIV

New Genera and Species of Fishes from Tortugas, Florida

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and

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With one plate and twenty-eight text figures

[ISSUED SEPTEMBER 14, 1940]

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New Genera and Species of Fishes from Tortugas, Florida

The new genera and species of fishes described in these pages resulted from studies carried on for many years, principally at Tortugas, Florida, under the auspices of the Department of Marine Biology of the Carnegie Institution of Washington, by the late Dr. William H. Longley, whose untimely death occurred before he had fully completed a study of his collections and a manuscript embodying a complete account of his field observations. The present writer has made further studies of the Tortugas collections, and has endeavored to extract interesting facts from Dr. Longley's notes on those species not treated by him in his unfinished manuscript.

As it is not practicable to publish the main work immediately, the new genera and species (including several species of which short descriptions already have appeared) are described and figured[1] in this preliminary paper for the purpose of making them available to workers in ichthyology as soon as possible.

It will be noticed that some of the species herein described for the first time are credited wholly to Longley, others to Longley and Hildebrand, and still others to Hildebrand only. Those species that Dr. Longley had named and for which he had drawn up at least partial descriptions are credited to him alone. In other instances, if his notes or manuscript indicated he had recognized the fishes as new, but had not named them, or had not completed descriptions, responsibility is shared. In the remaining few cases no indication was found that Dr. Longley recognized the fishes as new. For those few species I take full responsibility. S. F. H.

Promyllantor Alcock, 1890

The genera of the family Congridae stand in need of much more study, as pointed out by Parr (Bull. Bingham Oceanog. Coll., vol. 3, art. 5, 1932, pp. 19–31) and by Reid (Smithsonian Misc. Coll., vol. 91, no. 15, 1934, pp. 1–4). There seemingly can be no doubt, however, that the specimens from Tortugas described herein belong to that group which has been assigned to *Promyllantor* by Reid and others. Though I have seen no specimens of the type species of this genus, I have examined the type specimens of *P. alcocki* Gilbert and Kramer, with which the specimens at hand agree in dentition, except that the blunt, almost pavement-like teeth on the roof of the mouth extend farther back, that is, almost opposite the posterior angles of the mouth (see insert, fig. 1). The posterior nostril in the specimens from Tortugas, however, is situated in front of the eye, instead of being placed inward of the eye as in *P. alcocki*. In the specimens in hand, as in *P. alcocki*, the tail is considerably longer than the head and trunk. S. F. H.

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[1] The pen-and-ink drawings used herein were prepared by Mr. Andrew Pizzini unless otherwise stated.

Promyllantor schmitti Hildebrand, n. sp.

Dr. Longley's collection from Tortugas contains 5 specimens, ranging in length from 172 to 283 mm., which are listed in his field notes as having been taken south of Tortugas while dredging at a depth of 300 to 350 fathoms. Nothing further is to be found in Dr. Longley's notes. After devoting considerable time to the examination of these eels and the study of the literature, I have concluded that they are new. The species is named in honor of Dr. Waldo L. Schmitt, of the U. S. National Museum, who collected many of the deep-sea fishes included in the Tortugas collection while engaged chiefly in the collection of invertebrates.

The type, 280 mm. long (fig. 1), bears catalogue number 107323 in the U. S. National Museum collection. Body somewhat compressed anteriorly, strongly compressed posteriorly; greatest depth about 20.0 in total length, 7.5 in head and trunk; head deeper than broad, about 7.3 in total length, 2.7 in length from tip of snout to vent; snout rather blunt, spongy, projecting prominently beyond premaxillary, about 3.0 in head; eye moderately large, entirely lateral, its longest diameter fully equal to interorbital, 6.9 in head, 2.2 in snout; distance from tip of snout to origin of dorsal 1.9 in head and trunk; anterior nostril near lateral edge of soft, spongy, projecting snout, well in advance of premaxillary; posterior nostril elliptical, in front of about middle of eye, its margin entire; cleft of mouth not quite to below anterior margin of eye; tongue largely free. Teeth in jaws in bands, those in upper jaw pointed, exposed anteriorly when mouth is closed; those in lower jaw partly blunt; no canines; roof of mouth largely covered with blunt, almost pavementlike teeth, extending backward almost as far as cleft of mouth, band widest posteriorly (see insert, fig. 1). Gill clefts lateral, somewhat shorter than greatest diameter of eye; pores, other than the three slits on each side in upper lip, not prominent, those in lateral line too indefinite to be counted; vent situated far in advance of mid-length, distance from tip of snout to vent 2.7 in total length. Origin of dorsal about length of pectoral behind tip of latter, a little nearer vertical from vent than tip of snout, this fin confluent with caudal and anal, rays somewhat indefinite, about 230; caudal rather long, pointed, about as long as snout; anal similar to dorsal, with about 200 rays; pectoral behind and a little dorsal to gill opening, small, about 5.5 in head.

Color uniform brownish, tip of snout paler; gape and gill opening conspicuously surrounded with black; a longitudinally elongate dark area at shoulder above and in advance of gill slit; caudal, dorsal, and anal fins posteriorly dusky.

The following proportions show the variations in the type and paratypes: snout to vent 2.7 to 2.8 in total length; head 7.3 to 7.75 in total length, 2.7 to 2.8 in length from tip of snout to vent; depth about 18 to 24 in total length (small specimens slenderest), 2.5 to 3.1 in head; eye 5.9 to 6.9 in head, 1.4 to 2.2 in snout; snout about 2.9 to 3.8 in head; tip of snout to origin of dorsal 1.9 to 2.0 in distance from snout to vent; pectoral 5.0 to 6.4 in head. Vertebrae 37 anterior to vent and 93 posterior to it in a paratype.

In addition to the Tortugas material, there is at hand a specimen, 120 mm. long, taken in the Gulf Stream off Key West, Florida, in 304 fathoms (U.S.N.M.

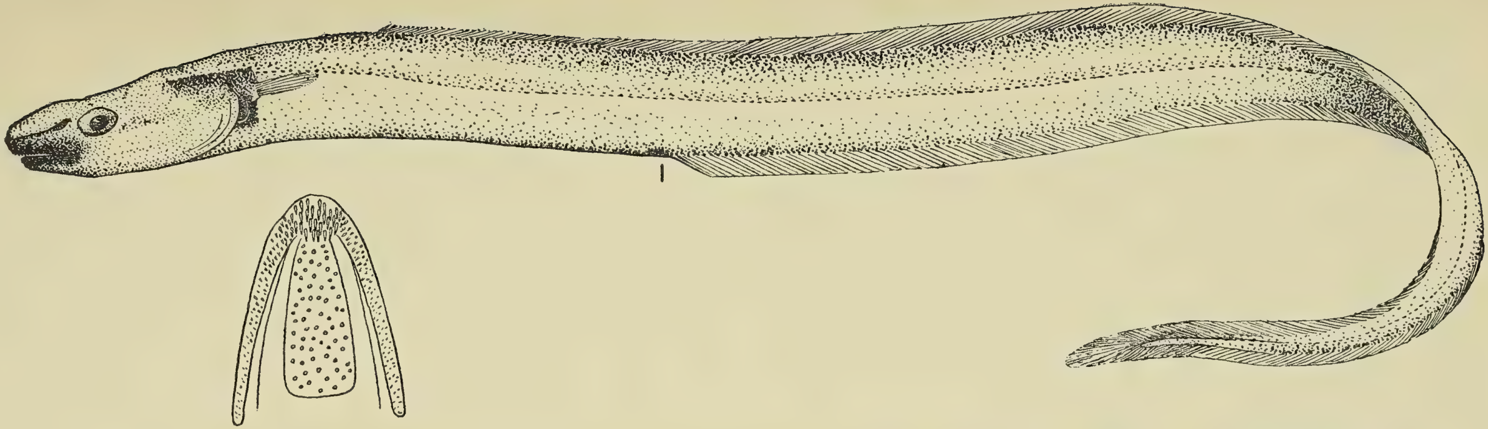

Fig. 1. *Promyllantor schmitti* Hildebrand, n. sp. From the type, 280 mm. long. Insert shows the teeth in upper jaw and on roof of mouth.

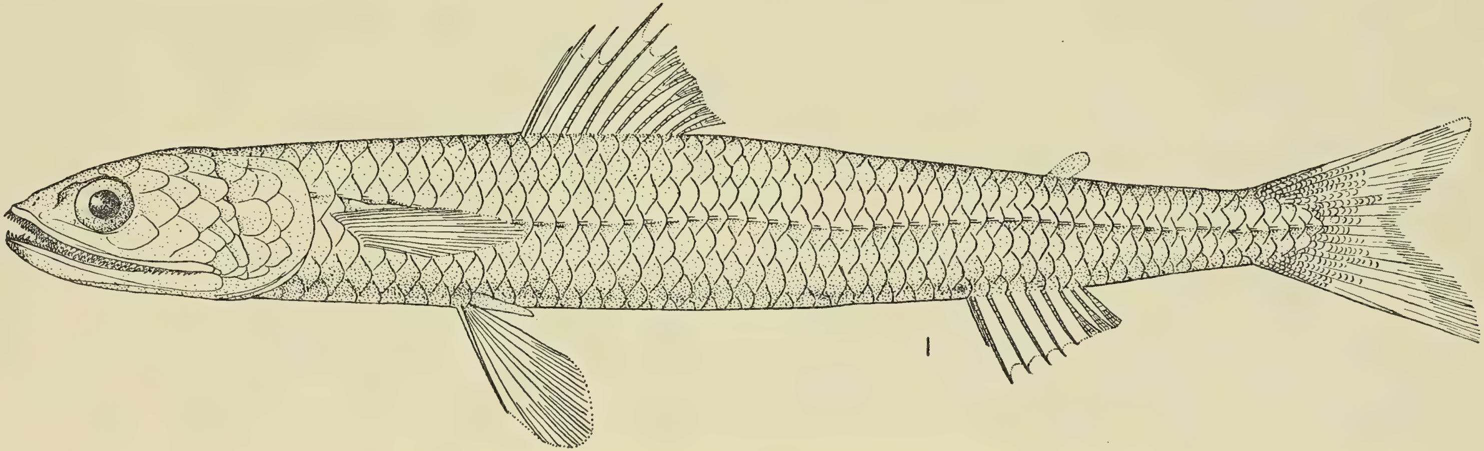

Fig. 2. *Saurida normani* Longley. From the lectotype, 380 mm. long.

No. 73270), which undoubtedly belongs to this species. This specimen agrees perfectly with Tortugas specimens of about the same size, even as to color markings. In this fish, as in those of similar size from Tortugas, black dots are scattered over the sides, forming three indefinite rows: one along the back, another just above the lateral line, and the third very irregular one just above the base of the anal, with more dots of the same size on the abdomen. In addition, four groups of smaller dark dots occur on the side: one below the lateral line slightly posterior to the origin of the dorsal, another just above the vent, and two on the tail. These dots are missing in the larger specimens, which are profusely and uniformly punctulate.

This species differs from *Pseudoxenomystax dubius* Breder in having a proportionately longer snout, smaller eye, smaller mouth, and more posteriorly inserted dorsal. From *Ariosoma perturbator* Parr it differs in the larger eye; in having the posterior nostril wholly in front of the eye, instead of partly above it; in the more posterior origin of the dorsal; in the longer and more pointed caudal fin; in the larger band of teeth on the roof of the mouth; and in having fewer vertebrae. The dentition of *Promyllantor schmitti* is very similar to that of *P. alcocki* Gilbert and Cramer, but the teeth on the roof of the mouth extend much farther back in the former. Furthermore, *P. alcocki* has the posterior nostril dorsal to the eye, and its vertebrae are more numerous (169). It agrees with *Congrosoma evermanni* Garman in the position of the posterior nostril, but it has a notably smaller mouth, a proportionately smaller head, and different color.
S. F. H.

Saurida normani Longley

Saurida normani Longley, Carnegie Inst. Wash. Year Book No. 34, 1935, p. 86—south of Tortugas, Florida, 60 to 100 fathoms.

To facilitate comparison, the description of this species is cast in the form adopted by J. R. Norman in his "Revision of lizard-fishes" (Proc. Zool. Soc. London, 1935, pp. 99–135). To him in appreciation of that work it is dedicated. It is not uncommon in the type locality.

Depth of body 6.0 to 9.0 in length to base of caudal; length of head about 4.1 to 4.3. Snout broader than long, about as long as eye, diameter of which is 4.4 to 5.0 in length of head and about equal to interorbital width. (One specimen with an exceptionally large eye, 3.8 in head, has not been included, as it may be abnormal.) Adipose eyelids of moderate width. Lower jaw a little shorter than upper, not visible from above when mouth is closed; greatest width of mandibles about 3.5 in head; length of premaxillary 1.4 in head. Teeth all pointed; outer bands of palatine teeth in two rows, the inner ones in a band; no teeth on head of vomer, and none to several on each side close to anterior of palatine series; teeth on tongue and basipharyngeals more or less in bands. Scales in general with spinous, almost denticulate borders; 52 or 53 in lateral line; 4 complete rows between middle of dorsal and lateral line, 14 to 16 between occiput and dorsal origin. Vertebrae 49 (about 31 + 18). Dorsal 11 or 12, counting last double ray as one, longest ray about equal to postorbital part of head and third of eye, and about 3.0 times as long as last ray; anal 9 or 10, counting last double ray as one, its origin about 3.1 times as far from head as from base of caudal; pectoral with 13 rays, extending much beyond base of pelvics, 1.3 to 1.5 in head; pelvic a little shorter, inserted well in advance of origin of dorsal and about 2.75 to 3.0 times as far from base of caudal as from tip of lower jaw; axillary scale long, pointed. Caudal peduncle not depressed, with slightly more distinct lateral keel than on scales of lateral line throughout its length. W. H. L.

The foregoing account, exclusive of the proportions and counts, which are based on specimens in the Tortugas collection, now in the U. S. National Museum, was prepared by Dr. Longley. I find the following concerning the color in his field notes, based on a specimen "375 mm. long" (380 mm. my measurement), which is the largest one in the collection: "Gray above, pale blue spots on one to two rows of scales on upper part of sides above the lateral line; sides and belly silvery with six marginal dots." Preserved specimens are grayish above and somewhat silvery underneath, with the base of both lobes of the caudal dusky.

The collection contains 9 specimens, ranging in length from 200 to 380 mm. This species evidently reaches a much larger size than *S. brasiliensis,* and it appears to inhabit rather deeper water. The specimens, so far as I have been able definitely to identify the records with specimens of this species, were taken in 60 to 100 fathoms.

Dr. Longley apparently did not designate a type. However, he referred to a specimen 375 mm. long both in his original description (see citation above) and in his notes, stating definitely in the latter that it bears tin tag no. 100, and that it was taken in 60 fathoms in an otter trawl, south of Tortugas. Though the specimen bearing tin tag no. 100 is 380 mm. long according to my measurement, there can be no doubt that it is the same fish. This is by far the best specimen in the collection and probably was intended as the type by Dr. Longley, but he seems to have overlooked designation. Therefore, the duty of making the designation falls to me. It becomes U.S.N.M. No. 107330 (fig. 2).

Dr. Longley (see citation above) said: "Differs from known Atlantic species by short lower jaw; from *S. suspicio* by long pectoral; from *S. caribbaea* by larger eye, fewer anal fin supports, fewer scales in lateral line; from *S. brasiliensis* and *S. parri* by smaller scales; has fewer pectoral rays than Indo-Pacific forms except *S. gracilis,* from which it differs in squamation." From *S. tumbil* this species seems to differ in having fewer but longer pectoral rays; shorter lower jaw; no teeth on head of vomer; and more posteriorly inserted anal. S. F. H.

Apogon quadrisquamatus Longley

Apogon quadrisquamatus Longley, Carnegie Inst. Wash. Year Book No. 33, 1934, p. 257—Tortugas, Florida.

The proportional measurements of the type are: Total length 78 mm.; standard length 62 mm.; depth 23 mm. (2.7); head 25 mm. (2.5); eye 8.0 mm. (3.1); and snout 5.0 mm. (5.0).

D. VI-I, 9½; A. II, 8½. Scales 2-24 or 25-7, all ctenoid except the 4 in the median row before the first dorsal and the anterior in the submedian row on either side of the nape; on either side of the caudal peduncle scales of nearly uniform size and very regular arrangement are in 3 rows above and 3 below the lateral line in addition to the median dorsal and ventral series. Gill rakers 11 or 12 on lower limb of first arch. Teeth in a villiform band in each jaw; similar teeth on vomer, and on palatines in the young. Posterior margin of preopercle rather strongly serrate. Color reddish, with yellow on the median fins, soon disappearing, no dark markings. A plain pale fish in spirits.

Common at Tortugas at the depth of 10 fathoms. Among known Atlantic species it is unique in details of squamation.

As "*Amia binotata,* var. sans tache" this species was sent by Poey from Havana to the Museum of Comparative Zoölogy. He also sent 9 specimens to Washington, which in the *Fishes of North and Middle America* (pt. I, 1896, p. 1109) Jordan and Evermann confused with *A. pigmentarius.* With a specimen of true *pigmentarius* and one of *Chilodipterus affinis* they are registered in the U. S. National Museum under numbers 37422, 37510, and 37514.

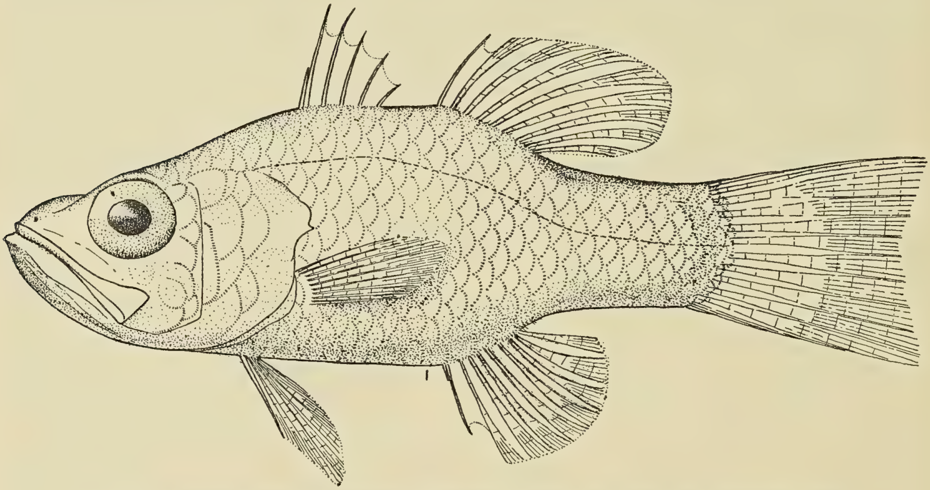

FIG. 3. *Apogon quadrisquamatus* Longley. From the lectotype, 75 mm. long.

It occurs in the Bahamas; the Museum of Comparative Zoölogy has a specimen (No. 27936) collected by the *Blake* in 27 fathoms, off Flanegan's Passage; the U. S. National Museum has another from near Mangrove Key; and there are 4 specimens, with 11 of *Apogon maculatus,* in the U. S. National Museum under number 39151 in a lot registered as comprising one specimen taken by the *Albatross* in 27 fathoms, between Tortugas and Tampa Bay. W. H. L.

Though Dr. Longley gave the length of 78 mm. (62 mm. standard length) for his type in his original description (see citation above), I find no specimen of exactly that length in his collection, and none labeled "type." Therefore, the duty of selecting a type falls on me.

The Tortugas collection contains 26 specimens, ranging in length from about 30 mm. (caudal fin broken) to 75 mm., which apparently are of this species. I designate the largest one as lectotype (U.S.N.M. No. 107325; fig. 3), though it may well be Dr. Longley's type, as the difference in the length shown may have resulted in part from shrinkage in preservative and in part from a difference in the methods of measuring used by the two workers. The following measurements (proportions enclosed in parentheses) and counts based on the lectotype are strikingly close to those given by Dr. Longley for his type (see first paragraph): Total length 75 mm.; standard length 59 mm.; head 25 mm. (2.35); depth 23 mm. (2.55); eye 7.9 mm. (3.2); snout 5.5 mm. (4.55); interorbital 5 mm. (5.0); maxillary 13.3 mm. (1.88); caudal peduncle 10.5 mm. (2.4); pectoral 15 mm. (1.67). D. VI-I, 9 (or 9½ if the last partly divided ray is counted as 1½, as Dr. Longley has done); A. II, 8; scales 2-25-6, 3 rows above and 3 below lateral line (in addition to a median dorsal and ventral series) on caudal peduncle, 4 in median series before dorsal; and gill rakers on lower limb of first arch 12 on left and 13 on right side. S. F. H.

Apogon pseudomaculatus Longley

Apogon pseudomaculatus Longley, Carnegie Inst. Wash. Year Book No. 31, 1932, p. 301—Tortugas, Florida. (Notes, but no description; no type designated.)

D. VI-I, 9½; A. II, 8½. Gill rakers 5 + 13 on first arch, the upper 2 and lower 3 very short. Squamation very regular; little difference in size in scales of one region; scales all ctenoid, 3-24 or 25-8, counting longitudinal series to base of caudal; 8 scales before dorsal; scales on caudal peduncle except in anal axil in 3 rows above and 3 below lateral line, median series excluded. In the median dorsal series on the caudal peduncle the scales are contiguous, in the median ventral not always so. Preopercular border weakly denticulate.

Color in life reddish with a black spot of half the diameter of the pupil at the base of the opercular spine. A black spot on each side below dorsal rays 7 to 9, and another above the lateral line near the base of the caudal. The second dorsal, the anal, and the lobes of the caudal fin black-tipped, but not so sharply black in the full-grown as in the young.

This is the species referred to incidentally by Evermann and Marsh in their description of *Apogon sellicauda,* which is *Apogon maculatus* (Poey). It is represented in the U. S. National Museum by 4 specimens (Nos. 58878–9) from Katama Bay, Massachusetts; 1 (No. 25351) from the stomach of *Centropristes* sp., caught 12 miles off Charleston, South Carolina; and 7 or more (No. 29151) taken by the *Albatross* between Tampa Bay and Tortugas in 27 fathoms. In the Museum of Comparative Zoölogy are 2 specimens (No. 2933) from Key West, Florida. The type, a specimen 57 mm. long, from Tortugas, Florida, is deposited in the U. S. National Museum.

As indicated by the above locality records, this species seems to occur more commonly than *A. maculatus* in deep water, and perhaps ranges farther north. At Tortugas a few specimens have been collected from beneath lumps of coral on Bird Key flats in company with *A. maculatus*. One 75 mm. long, with a brood of developing eggs in its mouth, was taken with the trawl in the 10-fathom channel east of White Shoal on June 26, and another in 40 fathoms, south of the Tortugas group, on August 17. W. H. L.

Dr. Longley did not designate a type when he proposed the name for this species (see citation above), but he did designate one in a foregoing paragraph. A specimen (U.S.N.M. No. 107324) labeled as the type in Dr. Longley's own handwriting is contained in the collection, though now it has a length of only 54 instead of 57 mm., the length stated above. There can be little doubt that this is the specimen selected as type by Dr. Longley, and may be so recognized (fig. 4). The difference in length probably is the result of shrinkage in preservative. There also are 17 paratypes, 22 to 80 mm. long, in the collection.

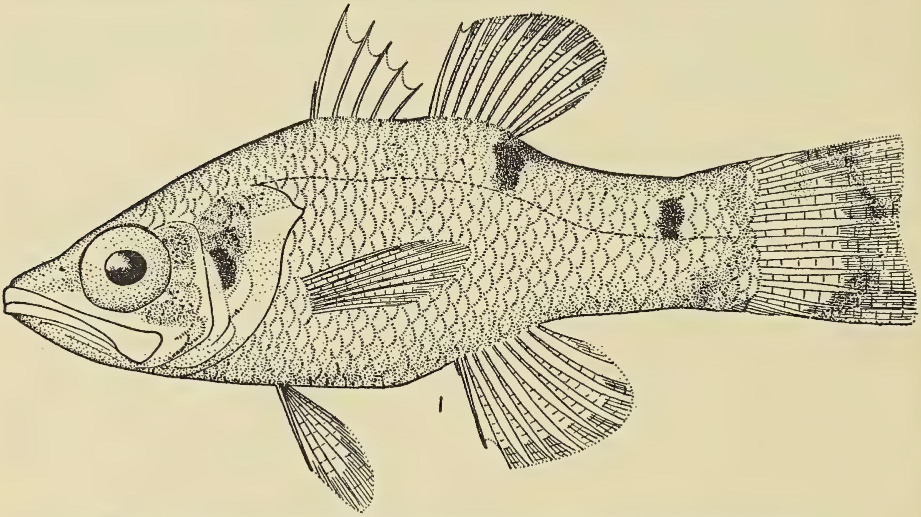

FIG. 4. *Apogon pseudomaculatus* Longley. From the type, 54 mm. long.

This species, among preserved material, is most readily separated from *A. maculatus,* to which it is rather closely related, by the smaller and more sharply defined black spots, one on the upper part of the side below the base of the second dorsal, and the other on the caudal peduncle, at the base of the upper lobe. The latter is not a saddle-like blotch as in *A. maculatus,* but a definite roundish spot. This species is distinguished, furthermore, by the black tips of the lobes of the dorsal and anal. According to Dr. Longley's account the tips of the caudal are also black; as this fin is more or less frayed in the specimens at hand, the black is no longer visible. Dr. Longley has shown differences in the scale counts. These must be carefully made, in the same place and same manner, or the differences will not be evident. S. F. H.

Apogon planifrons Longley and Hildebrand, n. sp.

The type (U.S.N.M. No. 107326; fig. 5) is about 75 mm. long (caudal fin damaged), standard length 59 mm., Tortugas, Florida.

Dr. Longley recognized an undescribed *Apogon* in his collection, for which he proposed the name *planifrons* in his manuscript. I have restudied the specimens, and am in agreement that two specimens in the Tortugas collection seem to be representatives of a hitherto undescribed species. However, I am in disagreement with Dr. Longley in regard to a specimen in the U. S. National Museum (No. 37529) from Cuba, which he identified as this species, and had tentatively selected as type, presumably because it is in better condition than either of the two Tortugas specimens at hand. The specimen selected by Dr. Longley as type certainly is different, as is shown by the deeper body, the deeper and less depressed head and snout, the more strongly serrated preopercular margin, apparently fewer scales in advance of the dorsal, fewer gill rakers, and the shorter pectoral fin with fewer rays. In all these respects the Cuban specimen agrees with those from Tortugas identified as *binotatus*. Evidently Dr. Longley had not found the opportunity of comparing his Tortugas specimens directly with the Cuban one.

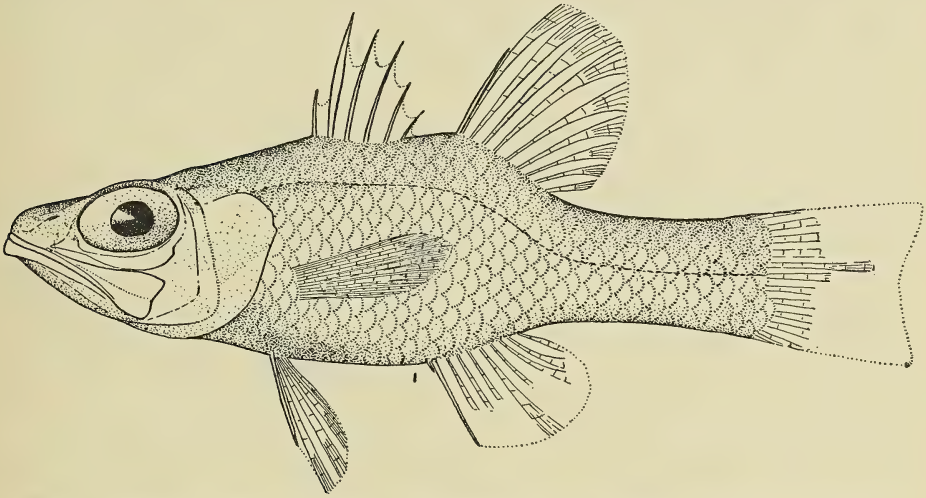

FIG. 5. *Apogon planifrons* Longley and Hildebrand, n. sp. From the type, 75 mm. long.

The two specimens at hand are not in very good condition, as already indicated. Unfortunately the better one, which I have designated as type, is without definite locality label and must be listed merely as having been taken at Tortugas. The paratype has more specific data, having been taken in an otter trawl in 205 to 221 fathoms. It seems probable that the type, too, was taken in rather deep water, though I have failed to find another *Apogon* listed in Dr. Longley's notes from deep water. However, *Hypoclydonia* (= *Synagrops*) is listed from the deeper hauls several times. It seems possible that this species, because of its slender form, escaped notice in the field and was wrongly listed under the genus named. Neither have I found any specimen listed from shallow water that I could identify with the one in hand. It can only be stated here that *A. planifrons* seems at times at least to inhabit rather deep water, whereas the other species of the genus included here was taken only in comparatively shallow water.

The following description is based on the type: Body rather elongate, only moderately compressed, depth 3.5 in standard length; caudal peduncle 2.9 in head; head low and long, depressed above, cavernous over snout and anterior half of interorbital space, 2.5 in standard length. Eye moderately large, 3.2 in head; snout low, as wide as deep at anterior margin of eye, 4.6 in head; interorbital flat, except for slightly elevated orbital rims, 4.9 in head; mouth large, oblique; lower jaw included at sides, projecting anteriorly where upper jaw is indented; maxillary broad posteriorly, reaching beyond pupil but not quite to posterior margin of eye, 1.8 in head. Teeth in villiform bands on jaws, vomer, and palatines; those on anterior part of jaws somewhat enlarged, but not canine-like; band on upper jaw prominently interrupted anteriorly, laterally mostly exposed if mouth is closed. Margin of preopercle feebly serrate, more feebly than in *A. binotatus,* to which this species is related, no serrations at lower posterior angle; opercular spine rather small; gill rakers rather numerous, long and slender, longest ones fully as long as pupil, 17 (including rudiments) on lower limb of first arch. Scales large, loosely attached (lost in part), strongly ctenoid, apparently 8 (pockets) in advance of dorsal, 25 in lateral line, on caudal peduncle 3 rows above and 3 below lateral line for the most part, with a median series above and below. Dorsal fins well separated, with VI-I, 9 rays, 2d spine stronger than 3d, but less so than in *binotatus,* 2d almost reaching tip of 3d if deflexed; anterior soft rays of second dorsal high, about as long as caudal peduncle, making the fin falcate; caudal broken, probably somewhat emarginate as in related species; anal with II, 8 rays, beginning very slightly in advance of second dorsal and almost coterminal with it, similar to second dorsal, but probably not quite as high (some of rays damaged), 2d spine strong; ventral fins with I, 5 rays, inserted in advance of pectorals and only slightly behind vertical from preopercular margin, reaching origin of anal; pectoral long, with 12 rays, reaching opposite middle of base of anal, 1.5 in head.

Color in alcohol pale, with dark points on sides of head. A band of dark chromatophores extending from posterior rays of second dorsal to posterior rays of anal; a similar band at base of caudal. These bands no doubt were more distinct when the specimen was fresh, as Dr. Longley stated in his notes that its color and pattern closely resemble those of *binotatus.*

The paratype is without scales, and the head and fins have been damaged. The following proportions and counts can be given as only fairly accurate: Depth in standard length 3.9; caudal peduncle 2.9 in head; head 3.1 in standard length. D. VI-I, 10; A. II, 8; gill rakers 18 (including rudiments) on lower limb of first arch. It clearly agrees with the type in the elongate body; in the long, low head, depressed above; and in the numerous gill rakers, which appear to be characteristic of the species. The eye appears to be larger and the snout correspondingly shorter in the paratype. Because of the imperfect condition of the specimen it is not possible to determine the exact proportions.

The relationship of this species, as already indicated, clearly is with *A. binotatus,* from which it differs in the more elongate body; lower, depressed head and snout; more weakly serrate preopercular margin; apparently more numerous scales in advance of dorsal; more numerous gill rakers; and longer pectoral fin, which also seems to have 2 more rays. Furthermore, the 2d dorsal spine seems less enlarged. S. F. H.

Prionodes notospilus (Longley)

Serranus notospilus Longley, Carnegie Inst. Wash. Year Book No. 34, 1935, p. 87—south of Tortugas, Florida.

Common in 40 to 60 fathoms, south of Tortugas. Twenty-eight specimens, 48 to 95 mm. long, were taken in one haul at a depth of 40 fathoms; 84 specimens, 64 to 100 mm. long, in another haul at 60 fathoms. The *Blake* took it at 36 fathoms (lat. 24° 34′ N., long. 83° 16′ W.) near the type locality, and also at 84 fathoms (lat. 23° 13′ N., long. 89° 10′ W.) several hundred miles away. Her catch is in the Museum of Comparative Zoölogy (Nos. 27959, 28105, and 28106).

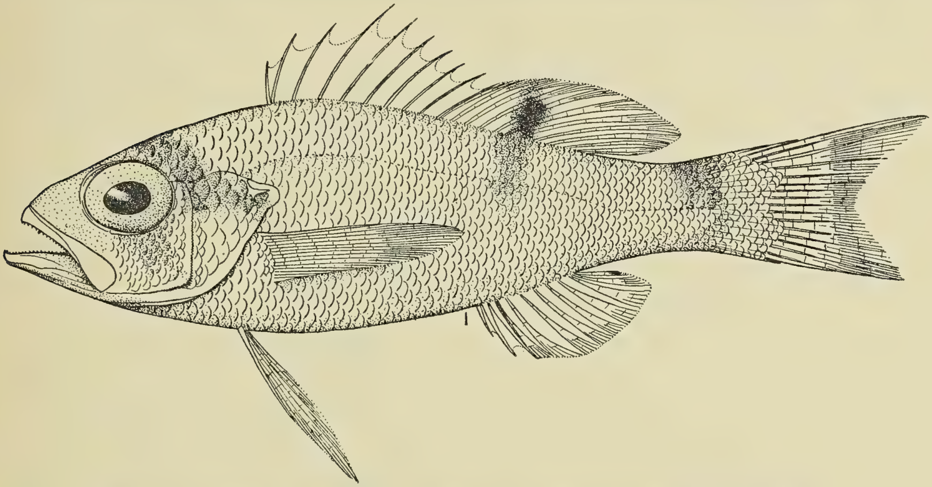

Fig. 6. *Prionodes notospilus* (Longley). From the type, 86 mm. long.

D. X, 12½; A. III, 7½; P. 15 or 16. Scales 6-47-15, 3 or 4 additional ones with pores on base of caudal; anteriorly extending into interorbital space to vertical of posterior pupillary margin; 6 oblique series on cheek, none cycloid. Teeth in upper jaw in a villiform band, the outer enlarged; in lower jaw the same, but in single series laterally; weak, villiform teeth on vomer and palatines; palatine teeth multiserial, the inner ones larger. Preopercular borders serrate, teeth at angle slightly enlarged; borders of subopercle and interopercle entire. Opercle terminating in 2 slightly diverging acute spines. Humeral spine rounded. Gill rakers 10 (and 3 rudiments) on lower limb of anterior arch, slight, the longest treble the interspace. Branchiostegals 7.

The proportional measurements of the type are: Total length 86 mm.; length to base of caudal fin 68 mm.; depth 22 mm. (3.1); head 22 mm. (3.1); eye 8 mm. (2.75); snout 6 mm. (3.66); interorbital width 3 mm. (7.3); 2d anal spine 7 mm. (3.15), slightly shorter and decidedly stouter than 3d.

Body slightly dusky above, paler below. A specimen 70 mm. long shows faint longitudinal striping, usually a pattern of bars. In this phase there is a faint band at the nape, and another below dorsal spines 4 to 6, both narrower below and inclined a little to the rear. Behind the second band and under the lateral line is a bright silvery area as in *P. phoebe,* which extends to the anus. From beyond the middle of the 3d dorsal ray a dark bar curves down to the base of the 7th, passing ventrally across the body beneath rays 5 to 8. The part on the fin is much darker than the rest and suggests the specific name. In large specimens this spot is fainter than in small ones. A last bar falls at the base of the caudal fin. The dorsal is cream-colored at its margin, and the tips of the caudal are orange.

Like a number of related species, this fish is normally hermaphroditic. The male section of the Y-shaped gonad covers its outer face on either side through its hinder half or third, the right and left lobes being united posteriorly across the ventral surface of the common trunk. Breeding occurs at least during August.

This species is distinguished from other west Atlantic species, except *tortugarum, fusculus,* and *atrobranchus,* by the few rows of scales on the cheek. From *tortugarum* it differs in the smooth sub- and interopercle; from *fusculus* in having only 15 or 16, instead of 18, pectoral rays; and from *atrobranchus* in the larger eye, color, and squamation. W. H. L.

Numerous specimens of this species are included in the collection. The type is entered in the U. S. National Museum under catalogue number 103765 (fig. 6).
S. F. H.

Prionodes nigropunctatus Hildebrand, n. sp.

On August 14, 1934 Dr. Longley took one specimen, 83 mm. long (63 mm. to base of caudal), in 30 fathoms. His notes, principally on color, were headed "*Prionodes tigrinus* (?)," with a line drawn through *tigrinus.* I find no other identification. According to current descriptions it certainly is not *P. tigrinus* (Bloch), as the scales in the lateral, as well as in the vertical, series are too few; the gill rakers are too numerous; and furthermore the color does not agree. Neither have I succeeded in identifying it with any other known species. Accordingly it seems necessary to recognize it as new, though the description of a new species with only one specimen at hand is unsatisfactory.

The type is registered in the U. S. National Museum under catalogue number 108877 (fig. 7). Head in standard length 2.6; depth 3.5. Eye in head 4.0; snout 3.5; interorbital 9.6; maxillary 2.4; caudal peduncle 2.7; pectoral 1.4; 2d anal spine 2.9. D. X, 10; A. III, 7; P. 13; branchiostegals 6; scales 6-55-17, about 10 rows on cheek; gill rakers 11 and 3 rudiments.

Body moderately compressed; profile anteriorly very gently convex; caudal peduncle rather deep; head low and long; snout moderately pointed; lower jaw projecting; maxillary broad, extending under middle of eye. Teeth present on jaws, vomer, and palatines; those in jaws anteriorly in bands, laterally mostly in a single series; upper jaw with 2 canines anteriorly at each side; lower jaw with 1 canine at each side anteriorly and 3 more laterally about halfway to angle of mouth. Gill rakers less than half length of pupil, moderately thick; preopercle rather strongly and evenly serrate to angle, lower limb mostly smooth; sub- and interopercle entire; opercle with 3 definite spines, the middle one longest; lateral line nowhere close to back, 4½ rows of scales between it and beginning of soft dorsal. Scales ctenoid, those in advance of dorsal considerably reduced, not extending forward on occiput quite to interorbital, extending somewhat on base of caudal, but not on the other fins. Dorsal fin not notched, spines strong, 4th to last of about equal length and shorter than soft rays; caudal lunate, with both angles produced; anal spines strong, 2d strongest, reaching tip of 3d if depressed, soft rays higher, nearly reaching base of caudal if depressed, base of fin coterminal with that of dorsal; ventral fins inserted very slightly in advance of pectorals, spine strong and scarcely half as long as produced soft rays; pectorals rather long with round margin, reaching tips of produced rays of ventrals, and falling short of reaching origin of anal by diameter of eye.

The color of the specimen when fresh was described by Dr. Longley as follows: "Ground color above is orange over the upper side of the head, brown under the spinous dorsal, grading to yellow posteriorly. On this, narrow blue lines run back through the interorbital space, two only as far as the dorsal origin, two beside the base of the spinous dorsal to its end, their anastomoses covering the interorbital and nuchal areas with a reticulum of blue about orange spots. In conjunction with these, other blue lines on the side of the head define an orange line from the margin of the upper jaw to the eye and rosy ones on the cheek below it and on the opercle. The blue line tangent to the eye below continues with minor irregularities to the base of the caudal. On the lower half of the side are six bars of yellow separated by blue ones of the same width in which two spots of yellow lie one above the other. Behind the eye are two blotches of the yellow ground color outlined with narrow brown lines. The iris is reddish orange like the adjacent parts of the head. It grows yellow toward the pupillary margin and has about a dozen dark sectors. On the dorsal fin is a black spot behind the tip of each spine and another behind the base of almost every one; on the soft dorsal a basal and submedian row of similar dark spots not so regularly arranged with reference to the fin supports. There are also several near the base of the upper lobe of the caudal. Dorsal and anal lobes and caudal straw

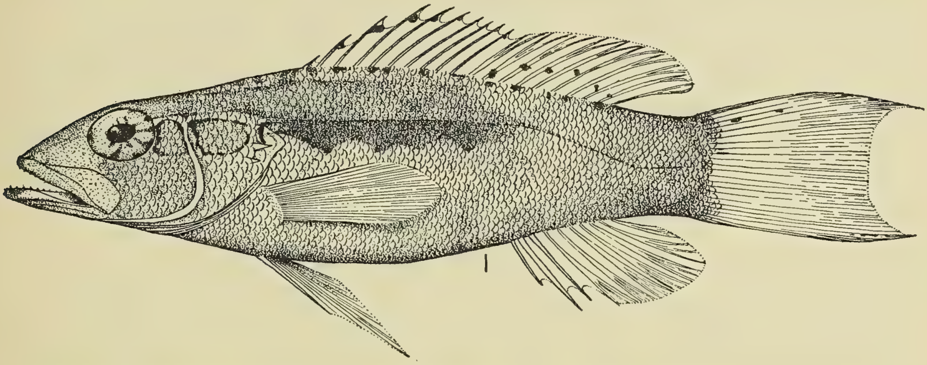

FIG. 7. *Prionodes nigropunctatus* Hildebrand, n. sp. From the type, 83 mm. long.

color. The tip of the lower jaw, a spot below the rictus, a vertical streak below the eye and in the angle of the preopercle correspond to elements in Evermann and Marsh's figure of *P. baldwini*. They carry enough dark chromatophores in state of contraction to suggest power of change of shade."

The colors as described have in large part been retained in the preserved specimen. The upper parts from the interorbital about to the beginning of the soft part of the dorsal, extending irregularly down to about the mid-line of the side, are brownish; most of the rest of the body is pale, but the chest and abdomen have a silvery sheen which seems to be rather usual in this group of fishes (*Prionodes* and *Dules*). The darker markings described by Dr. Longley, of course, remain. Two pale (orange in life) areas, outlined with dark brown lines, behind the eye are conspicuous, and the dark spots on the dorsal and the upper part of the base of the caudal remain prominent. The dark spots in the lower series are partly on the back and partly on the base of the dorsal fin. Except for the black spots mentioned, the fins are straw color in the preserved fish.

This species seems to be characterized especially by the unique color, the small number of rays in the soft part of the dorsal, the rather numerous rows of scales on the cheek, and the reduced scales on the nape, which do not extend forward even as far as the posterior rims of the orbits.

The abdomen was cut open for examination of the gonads, concerning which Dr. Longley noted, "Hermaphroditic; find mature eggs in this fish and the testicular area in the bisexual gonad." S. F. H.

Serranus tortugarum Longley

Serranus tortugarum Longley, Carnegie Inst. Wash. Year Book No. 34, 1935, p. 87—south of Tortugas, Florida.

Two specimens were secured in the otter trawl at 30 fathoms.

D. X, 11½ to 12½; A. III, 7½; P. 14. Scales in lateral line 50; 7 oblique series on cheek; all scales ctenoid. Interopercle scaleless, its margin, as well as that of the subopercle, serrate. Gill rakers on lower limb of first arch 19 to 21.

Color rufous orange above, becoming paler on sides. Back and side with nine narrow blue vertical bars, about the width of the pupil, mostly not extending below the lateral line. The first bar lies on the interorbital space, the second above the preopercular margin, the third above the opercular spine, the fourth under the 2d to 3d dorsal spines, the fifth under the 6th to 8th dorsal spines, the sixth under the 1st to 3d soft rays of the dorsal, the seventh under rays 6 to 8, the eighth at the end of the dorsal base, and the ninth on the caudal peduncle. Russet blocks on the back extend on the dorsal fin. Caudal fin finely cross-barred.

This fish is hermaphroditic. The male section of the gonad is readily recognizable by the texture. Spawning takes place at least in August. Specimens of about 80 mm. in length are sexually mature. W. H. L.

Dr. Longley did not designate a type. He had two specimens, which are now before me. These are respectively 78 mm. (standard length 62 mm.) and 73 mm.

(standard length 58 mm.) long. I have designated as lectotype the larger one, which is deposited in the U. S. National Museum under catalogue number 107329 (fig. 8).

The following proportions and counts made by me are based on the two specimens in hand. The ones based on the paratype are enclosed in parentheses: Head in standard length 3.0 (2.9); depth 3.2 (3.4); pectoral 3.8 (3.4); ventral 4.3 (4.0). Eye in head 3.1 (3.2); snout 4.1 (4.5); interorbital 4.4 (5.0); maxillary 2.4 (2.3). D. X, 11 (X, 12); A. III, 7 (III, 7); P. 13 (14); V. I, 5 (I, 5); scales 9-65-19 (8-62-18), 7 (6) oblique rows on cheek; branchiostegals 6 (6); gill rakers 20 (21).

Lateral line follows curve of back, and is not close to base of dorsal; opercle with 3 spines, middle one being largest; mouth moderately large, oblique, maxillary nearly reaching middle of eye. Teeth in narrow bands on jaws, vomer, and palatines; the outer ones in anterior part of upper jaw and laterally in lower jaw slightly enlarged. Gill rakers close together and rather long, longest being fully as long as pupil. Ventrals inserted under base of pectorals, and rather long and pointed, nearly reaching vent; pectorals with rounded outline, reaching a little beyond tips of ventrals; dorsal scarcely notched, though posterior spines are a little shorter than 4th and 5th, which are longest. The 2d and 3d anal spines are of nearly equal length and thickness. S. F. H.

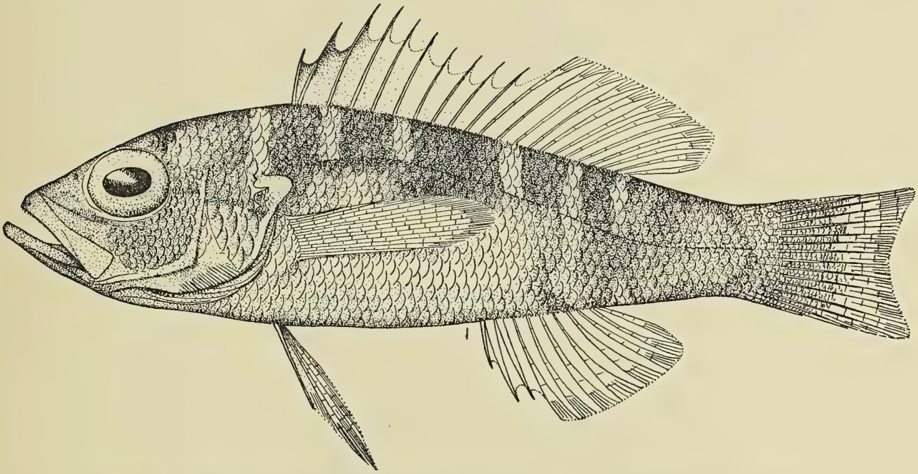

FIG. 8. *Serranus tortugarum* Longley. From the lectotype, 78 mm. long.

Serranus beta Hildebrand, n. sp.

A specimen having a total length of about 105 mm. (caudal slightly damaged) and standard length of 84 mm. is included in the Tortugas collection. There is no definite locality label with the specimen, and I have been unable to connect it with any of Dr. Longley's notes. However, it undoubtedly was taken south of Tortugas, in water of 30 or more fathoms in depth, with many specimens of related species.

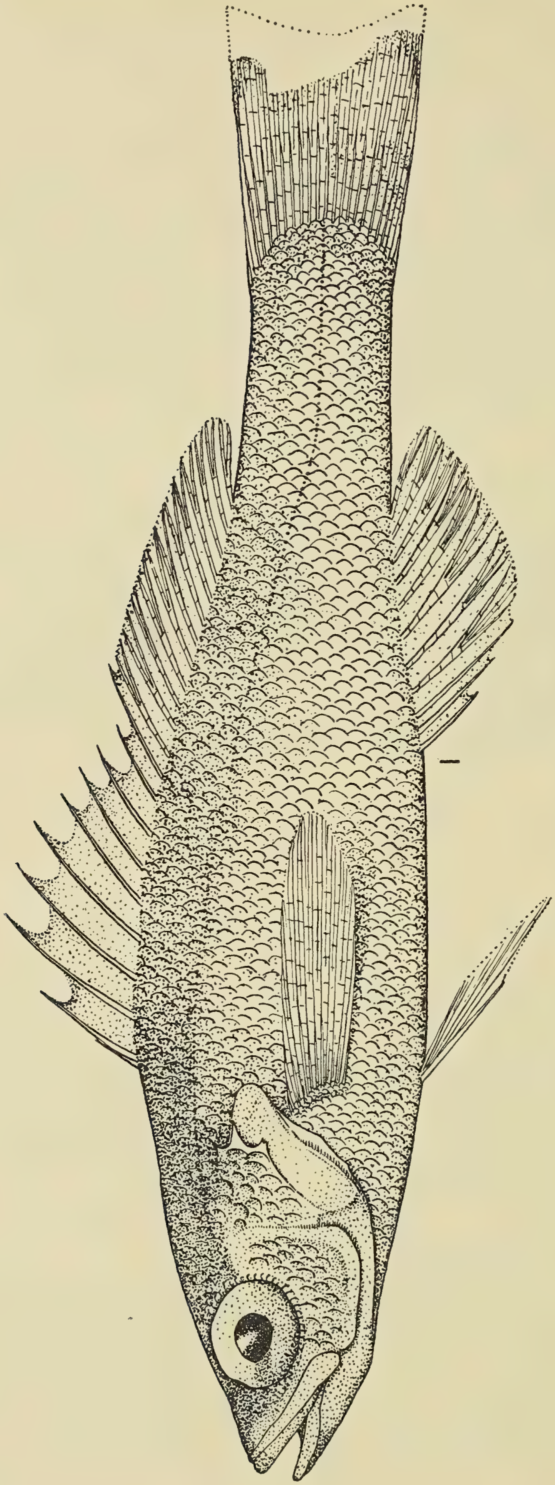

Fig. 9. *Serranus beta* Hildebrand, n. sp. From the type, 105 mm. long.

This fish is related to the specimens named *S. tortugarum* by Dr. Longley, but differs in color and several other respects, as will be shown subsequently. I am unable to identify it with any known species, and therefore am making it the type of a new species. The name *beta* is proposed because this is the second species from Tortugas of uncertain generic affinities included in this paper.

The type is deposited in the U. S. National Museum under catalogue number 108878 (fig. 9). Head in standard length 3.2; depth 4.4. Eye in head 3.4; snout 4.3; interorbital 4.5; maxillary 2.5; caudal peduncle 2.9; pectoral 1.2; 2d anal spine 4.3. D. X, 12; A. III, 7; P. 17; branchiostegals 6; scales 4-60-15 (lost in part, making counts somewhat uncertain), about 5 or 6 rows on cheek; gill rakers 25.

Body quite slender, compressed; profile anteriorly very gently convex; caudal peduncle rather slender; head low and long; snout moderately pointed; lower jaw scarcely projecting; maxillary rather narrow, rounded posteriorly, scarcely reaching middle of eye; teeth exceedingly minute, none enlarged, present on jaws and apparently in two small patches on roof of mouth; gill rakers about half as long as eye, rather close-set; preopercular margin serrate, serrae somewhat enlarged at angle; margin of sub- and interopercle serrate; opercle with 3 flat spines, middle one much the largest, lowest one bifurcate; lateral line for the most part following outline of back, but not running close to it, 3½ rows of scales between it and beginning of soft dorsal. Scales ctenoid, extending forward on interorbital, with indefinite outlines of scales on snout in advance of interorbital; posterior half of margin of orbit outlined with a modified partly embedded series of scales; scales present on subopercle, extending on caudal fin and to some extent on interradial membranes of the other fins. Dorsal fin not perceptibly notched, last spine somewhat longer than immediately preceding ones but a little shorter than 4th, which is longest, soft part a little higher than spinous; caudal apparently rather deeply lunate, exact shape indeterminable because of damage; anal small, very nearly coterminal with dorsal, spines rather weak, 2d notably shorter than 3d and scarcely as strong; ventral fins inserted very slightly behind base of pectorals, spine strong, somewhat more than half length of longest soft rays; pectorals moderately long, scarcely reaching beyond tips of ventrals, failing to reach origin of anal by diameter of eye.

General color brownish, with lower parts of head, chest, and abdomen silvery. No very definite markings anywhere. Back with about five or six indefinite dark areas, two areas behind eye slightly darker brown than ground color; tip of lower jaw brownish. Dorsal fin slightly brownish, with indications of a dark spot behind each spine and soft ray near the margin (the fin being somewhat frayed, this spotting cannot be seen distinctly behind some of the rays); caudal fin brownish with indications of darker cross shades; anal fin pale, with indications of white spots; ventrals and pectorals plain translucent, the latter with minute dark points on basal parts and a dark membrane in axil.

This species is related to *S. tortugarum,* with which it agrees in a general way in having the sub- and interopercle serrate, the gill rakers long and numerous, and the teeth relatively small. It differs, however, in the plainer color, the much more slender body, the fewer scales (especially in the vertical series), the even smaller teeth, the more numerous pectoral rays and gill rakers, the slightly more posterior insertion of the ventrals, and the notably smaller 2d anal spine.

S. F. H.

Pronotogrammus aureorubens Longley

Pronotogrammus aureorubens Longley, Carnegie Inst. Wash. Year Book No. 34, 1935, p. 88—south of Tortugas, Florida.

This fish is rather common south of Tortugas at depths of 100 to 200 fathoms, where 24 specimens, ranging upward to 290 mm. in length, were secured.

D. X, 15½; A. III, 8½; P. 16 or 17; scales 6-44 to 46-17, ctenoid, none in anterior half of interorbital space or on snout or maxillary; gill rakers long, slender, closely approximated, 28 or 29 on lower limb of anterior arch. Mouth oblique; upper jaw with wide median emargination receiving tip of lower; each side of emargination with several moderate canines, which when mouth is closed are outside dentary; as in related species, though much less noticeably, 1 or more lateral teeth rise above the others, while the rest in the upper jaw are in a narrow villiform band, wider anteriorly; teeth in lower jaw for the most part in a single series and weak, except for 1 or 2 on either side of the symphysis, behind which several depressed teeth point backward; very weak teeth on vomer and palatines, surface of which is scarcely more than granulated. Preopercle serrate on horizontal and vertical borders, the teeth near its angles sometimes larger than the others; subopercle and interopercle serrate on free border near their articulation; opercle with 3 spines, upper one blunt and remote, median one sharpest, median and lower ones sometimes compound.

Dorsal surface pinkish; scales yellow-margined; sides and belly silvery; iris golden. Dorsal and caudal fins yellow; pectorals pink; other fins pale.

The type (U.S.N.M. No. 101320; fig. 10) is 270 mm. long including caudal filaments, standard length 175 mm. The following proportions and counts are based on this specimen: Head in standard length 2.8; depth 2.85; pectoral 3.2; ventral 4.25. Eye in head 3.45; snout 3.9; interorbital 4.15; maxillary 2.2.; caudal peduncle 2.95. D. X, 14½; A. III, 8½; P. 16; scales 5-48-16, 2 complete rows between lateral line and middle of dorsal base, and 3 rows at base of last dorsal ray, 7 oblique rows on cheek; gill rakers 28; branchiostegals 6.

This species is nearest *P. eos* Gilbert from the Pacific coast of tropical America, from which it differs in having smaller scales, a more oblique mouth, and feebler dentition.

The sexes appear to be distinct. W. H. L.

In the foregoing account by Dr. Longley the paragraph pertaining to the measurements and counts based on the type was somewhat revised and enlarged by me after careful examination of the type. Although this species is placed in a different genus, it resembles *Hemianthias vivanus* greatly. The shape of the body, head, and mouth is virtually identical; the shape and posi-

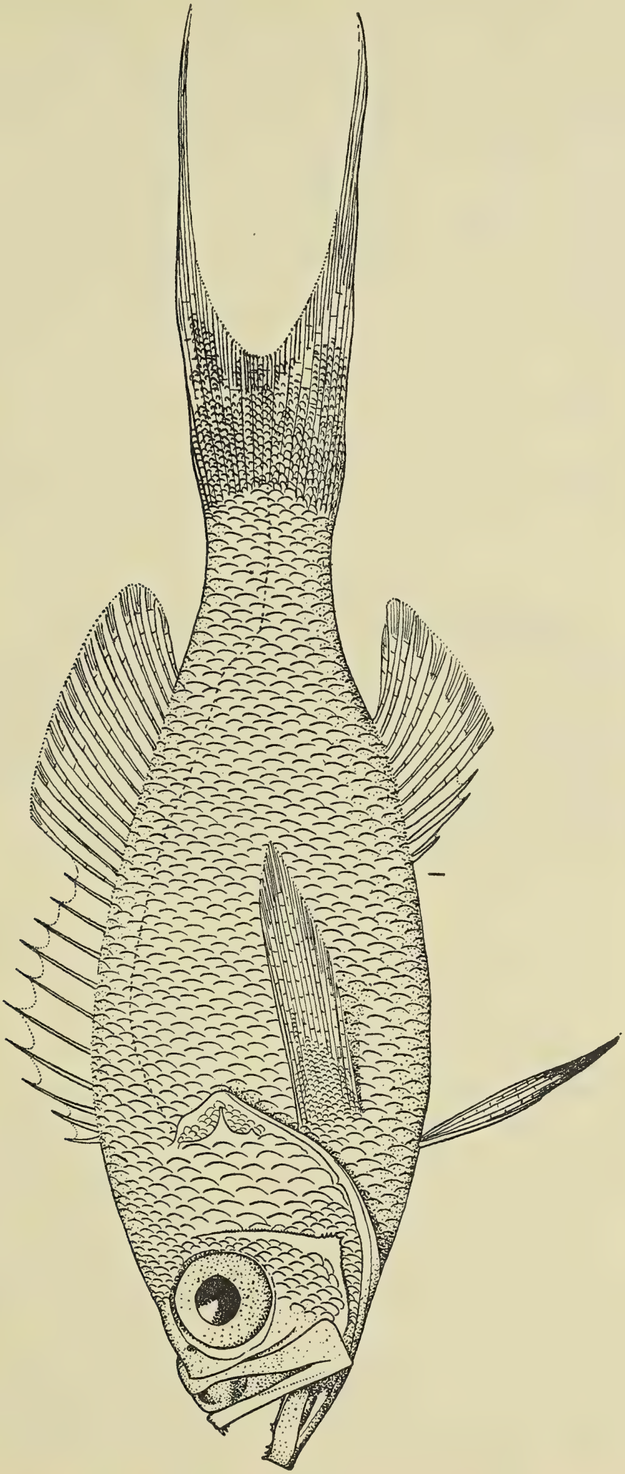

Fig. 10. *Pronotogrammus aureorubens* Longley. From the type, 270 mm. long.

tion of the fins, including the filamentous rays of the caudal and short filaments of the spinous dorsal, and the number of rays included in the fins are very close. The ventral fins are scarcely more posteriorly inserted than in *H. vivanus* (supposedly a generic character), but they do not develop filaments with age (which is scarcely evident in *H. vivanus* under a length of about 70 mm.). The lateral line in each species assumes an identical course, and in each species the gill rakers are long and equally numerous. However, *P. aureorubens* has a much larger eye; there is no single enlarged spine at the angle of the preopercle; and the entire lower limb is serrate. The margin of the subopercle, and sometimes the interopercle in part also, is serrate. The color of preserved specimens is plain pale in each species. S. F. H.

Rhegma brederi Hildebrand, n. sp.

A single specimen, 82 mm. long (standard length 65 mm.), is included in the Tortugas collection. There is no locality label with this fish. However, it evidently was picked up on the bird rookery, as is shown by bits of shell clinging to the various structures within the branchial chamber, and by its somewhat shriveled condition, undoubtedly caused by partial drying before it was found. The conclusion that it was dropped by a bird in feeding its young is strengthened by the similar appearance of other specimens picked up on the rookery.

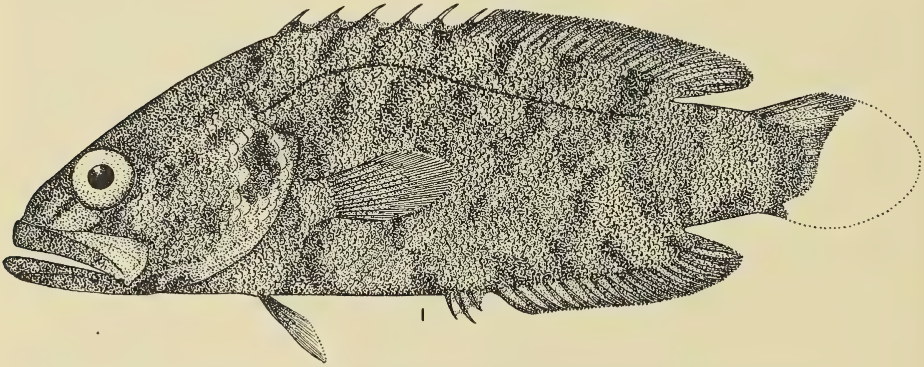

Fig. 11. *Rhegma brederi* Hildebrand, n. sp. From the type, 82 mm. long.

It is unfortunate that this imperfect specimen must serve as the type of a new species. However, aside from being hardened and somewhat shriveled, it is in fair condition. It retains its scales, and none of the fins except the caudal are damaged. Even that fin is not injured too severely to determine its shape. This specimen bears catalogue number 108865 in the U. S. National Museum collection (fig. 11).

Head in standard length 2.6; depth 3.0; dorsal base 1.6; anal base 3.1. Eye in head 6.0; snout 3.8; interorbital 12.5; maxillary 1.9; caudal peduncle 2.8; longest dorsal spine 4.1; longest anal spine 5.5; pectoral 1.5. D. VII, 21; A. III-17; P. 14; scales 50; gill rakers 9.

Body rather strongly compressed; dorsal profile anteriorly moderately steep and convex; ventral profile nearly straight anteriorly; head moderate; snout rather pointed, scarcely longer than eye; mouth large, slightly oblique; lower jaw scarcely projecting; maxillary broad, with a narrow supplemental bone, reaching far beyond posterior rim of orbit; teeth in bands on jaws, vomer, and palatines, some of the inner ones on anterior part of both jaws enlarged; preopercle with a strong spine originating near its upper angle and directed downward; opercular spines scarcely evident; gill rakers short, thick, spinose; scales ciliate, becoming smooth anteriorly, reduced in size on head, extending forward to anterior rim of orbit, present also on cheeks, gill covers, and bases of vertical fins; exposed part of each scale with many horizontal striae; lateral line ascending rapidly to under base of about 3d dorsal spine, then following contour of back to base of about 4th ray from end of dorsal, curved upward at tip, and ending without reappearing on caudal peduncle.

Dorsal and anal fins rather low, spines strong, those of anal nearly separate from rest of fin; caudal short, round; ventrals inserted a little in advance of pectorals, reaching about halfway to vent; pectorals notably longer, slightly pointed, reaching origin of anal.

Color very dark brown; a dark opercular spot present; sides with somewhat obscure irregular, vertically elongate dark spots, extending on bases of dorsal and anal, and tending to form reticulations.

This species is close to *R. thaumasium* Gilbert (in Jordan and Evermann, Bull. U. S. Nat. Mus., vol. 47, pt. 4, 1900, p. 3170) from the Pacific coast of Panama, from which it differs, however, in the absence of an appendage over the orbit, and in the absence of the lateral line on the caudal peduncle. It differs, also, in having some enlarged teeth in the jaws anteriorly, which *R. thaumasium* does not possess. It differs furthermore in having the interorbital definitely scaled, and in having a longer pectoral, which reaches fully opposite the origin of the anal, whereas it falls short of this point in *R. thaumasium*.

This fish is related also to *R. guineensis* Norman (Discovery Reports, vol. 12, pt. I, 1935, p. 10, fig. 2) from the Gulf of Guinea, from which it differs in the notably larger mouth, the maxillary reaching only slightly past the posterior margin of the orbit in that species, whereas it reaches far beyond the eye in the present one. The fish before me, also, seems to have a proportionately smaller eye, contained 4.9 times in the head, whereas the eye in *R. guineensis* is contained in the length of head only 4.0 times. It differs from that species, as it does from all others known, in the absence of a lateral line on the caudal peduncle.

From *Caribrhegma gregoryi* Breder (Bull. Bingham Oceanog. Coll., vol. 1, 1927, p. 44, fig. 22) from Glover Reef, off the Atlantic coast of Honduras, which Norman (see citation above) has referred to *Rhegma,* it differs in the absence of a tentacle over the eye, and in having more rays in the dorsal and anal, the formulae for that species being D. VII, 15; A. III-12.

As already pointed out, the present species differs from the other three species of the genus known in the absence of a lateral line on the caudal peduncle. Apparently the scales differ, also, in being ciliate instead of finely serrate, and in having horizontal striae on their exposed surface, which at least are not mentioned in the descriptions of the other species. Neither are dark spots or reticulations on the body mentioned in the accounts of the other species, though slightly evident in one small specimen among several of *R. thaumasium*.

I take pleasure in naming this species for Dr. Charles M. Breder, Jr., who described the first species of this genus, as understood here, to become known from the Atlantic Ocean, and who himself indeed collected specimens on the Bird Key rookery in the Tortugas. S. F. H.

Scorpaena dispar Longley and Hildebrand, n. sp.

A single specimen, with a total length of 112 mm., standard length 87 mm., was taken south of Tortugas at a depth of about 40 fathoms. It is listed in the U. S. National Museum under catalogue number 108867 (fig. 12).

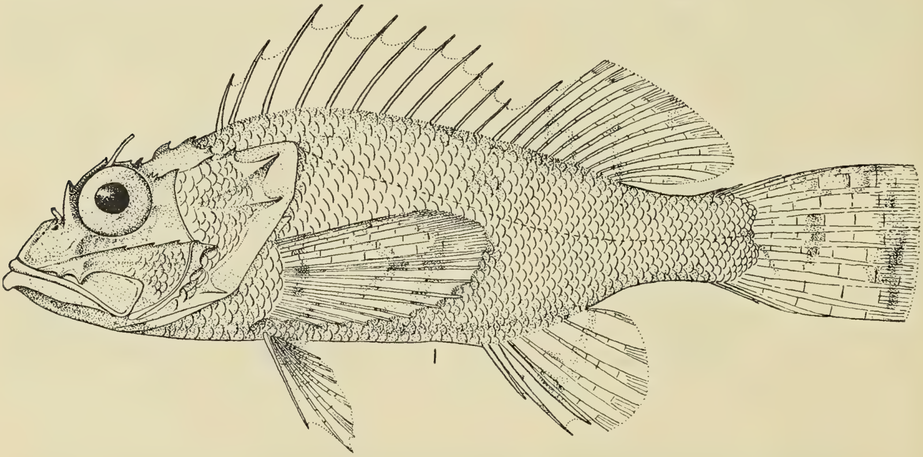

Fig. 12. *Scorpaena dispar* Longley and Hildebrand, n. sp. From the type, 112 mm. long.

Head in standard length 2.4; depth 3.2. Eye in head 3.9; snout 3.6; maxillary 2.1; interorbital 8.8; caudal peduncle 4.3; 4th dorsal spine 2.0; 2d anal spine 2.1; ventral 1.5; pectoral 1.3. D. XII, 10; A. III, 5; P. 19; scales 4-40-12; gill rakers 6 somewhat developed on lower limb of first arch.

Body rather elongate, compressed; head compressed, greatest width equal to depth at middle of orbit; profile from snout to dorsal convex, except for a depression in advance of eyes, and another at the rather prominent occipital pit; snout moderately broad; mouth large, oblique, maxillary not greatly expanded, reaching opposite posterior margin of pupil; lower jaw included at sides, but projecting slightly at tip. Teeth villiform, in rather broad bands in jaws, band in upper jaw broadly interrupted anteriorly; bands of similar teeth on vomer and palatines. Gill rakers very short, broad, and spiny. Scales moderately large, cycloid, extending forward to but not on interorbital, present on cheek and opercle, greatly reduced on chest. Spines on head rather short and thick; 1 pair between nostrils; 3 pairs on supraorbital ridge, 1 anteriorly and 2 posteriorly;

1 pair in advance of occipital pit, and 2 behind it; 2 rows of converging spines below the latter, the last one of these at apex of angle formed in beginning of lateral line; 1 blunt spine just behind opercular margin, about halfway between base of pectoral and lateral line; opercle with 2 strong divergent spines; preopercular margin with 5 spines, uppermost one with a supplemental spine at base, and in line with suborbital keel; preorbital with 3 strong spines, the first 2 antrorse; suborbital keel with 4 spines, exclusive of the preopercular one, which really forms the terminal one on this keel; a short dermal tentacle behind 1st supraocular spine, and a much longer one originating between bases of the 2 posterior supraocular spines; other dermal flaps on margins of preorbital and preopercle; some rather long, slender ones along lateral line, and smaller ones on scales on sides.

Dorsal fin moderately notched, 4th spine slightly longer than 3d and 5th, and about as long as longest soft rays, latter reaching base of caudal if deflexed; margin of caudal apparently slightly rounded (rays somewhat frayed); 2d anal spine stronger, but scarcely longer than 3d, and about as long as longest soft rays, latter not quite reaching base of caudal if deflexed; ventral fins reaching a little beyond vent; pectorals much larger, reaching origin of anal, uppermost 1 and lower 12 rays simple and median 6 branched.

The color of this specimen when fresh was described as follows by Dr. Longley: "Color red, much spotted with white under the head, streaked and vermiculated elsewhere in an intricate pattern. In all major color masses the dark is darker, the light lighter toward the margin. Iris with radiating lines. Rather regular narrow bars on pectoral and caudal; two rather conspicuous red bars on anal; ventrals red. No dark spots in pectoral axil or upon the side behind it. The fin itself with a few dark spots on its posterior face. The mouth almost entirely red within."

The preserved fish has faded to a straw color, being a little paler below than above. A few indefinite dark blotches remain on the head and body. The dark bars on the pectoral and caudal remain, though inconspicuous, and the anal also has indefinite dark bars. The dark spots on the inner surface of the pectoral remain rather prominent.

This species seems to be nearest *S. brasiliensis,* from which it differs, however, in having fewer scales, in having more numerous spines on the suborbital keel, and prominently in color. It differs from all the local species, so far as specimens have been available for examination, in having 3 definite spines on the supraorbital.

This specimen was found in the collection unidentified, and was listed in Dr. Longley's field notes as "*Scorpaena* sp. Compare with *grandicornis* and *albifimbria,*" which seems to show that he recognized it as unusual. S. F. H.

Neomerinthe tortugae Hildebrand, n. sp.

Three specimens of this species, 135, 200, and 245 mm. long, are included in the Tortugas collection. These were all taken in one haul with the otter trawl south of Tortugas, in 60 fathoms. These specimens, at least tentatively, had been identified by Dr. Longley as *Pontinus beanorum* Evermann and Marsh. From a comparison with the type of that species, however, several differences are evident. The scales especially are much smaller in the Tortugas specimens, wherein, as is shown subsequently, I have counted 60 to 65 in the lateral series, 6 or 7 rows between the lateral line and the origin of the dorsal, and 12 between the lateral line and the origin of the anal, whereas in the type of *P. beanorum,* counted in the same way, I found only 4-39-8. Furthermore, in the Tortugas specimens the scales extend forward on the interorbital and snout, covering also the cheeks and part of the maxillaries, becoming very small anteriorly but remaining strongly ctenoid, as on the body. In *P. beanorum* they do not extend forward on the interorbital, snout, and maxillary, and are large and scarcely ctenoid on the cheeks. The gill rakers, though equal in number, are longer in *P. beanorum.*

The two species agree in having some of the median rays of the pectorals divided, at least in adults, and therein differ from representatives of the genus *Pontinus.* However, the pectoral fins are narrow, the suborbital keel has 4 spines, the preorbital has 2 well-developed ones, and there is no occipital pit, in which respects both species agree with typical *Pontinus.*

The specimens generically would be identical with *Neomerinthe hemingwayi* Fowler (Proc. Acad. Nat. Sci. Phila., vol. 87, 1935, p. 41) if it were not for the peculiar spinous dorsal of the specimen on which that genus and species were based. The description and figure of *N. hemingwayi* show that the 1st dorsal spine is well separated from the others, and only 8 additional ones are present in the first part of the dorsal. If this condition is normal, which Fowler apparently was inclined to doubt, it would seem to be of generic significance. It is noticed that he did not consider it in that connection, basing his new genus rather on the branched rays in the pectoral, wherein it agrees with the Tortugas specimens now before me, and with the type of *P. beanorum,* which is also before me. Furthermore, the suborbital keel, as well as the preorbital, are similarly provided with spines, and the gill rakers are few and apparently rather short. Therefore, if the peculiar condition of the dorsal in Fowler's specimen on which he based the genus *Neomerinthe* is regarded as abnormal, the species herein described, as well as *P. beanorum,* apparently should be referred to that genus.

Neomerinthe, as here understood, differs from *Pontinus* in having some of the pectoral rays branched in the adult (though probably not in the young), wherein it agrees with *Helicolenus.* In the spiny suborbital keel it agrees with *Pontinus* and disagrees with *Helicolenus.* Finally, in the few, short, spinulate gill rakers it apparently differs from both related genera. Though Fowler's *Neomerinthe* is herein recognized, it seems highly probable that species will be found that will bridge the gaps now apparently separating this genus and *Pontinus* and *Helicolenus.*

The largest specimen, which has a total length of 245 mm., and standard length of 200 mm., has been selected as type. It is deposited in the U. S. National Museum under catalogue number 108871 (fig. 13).

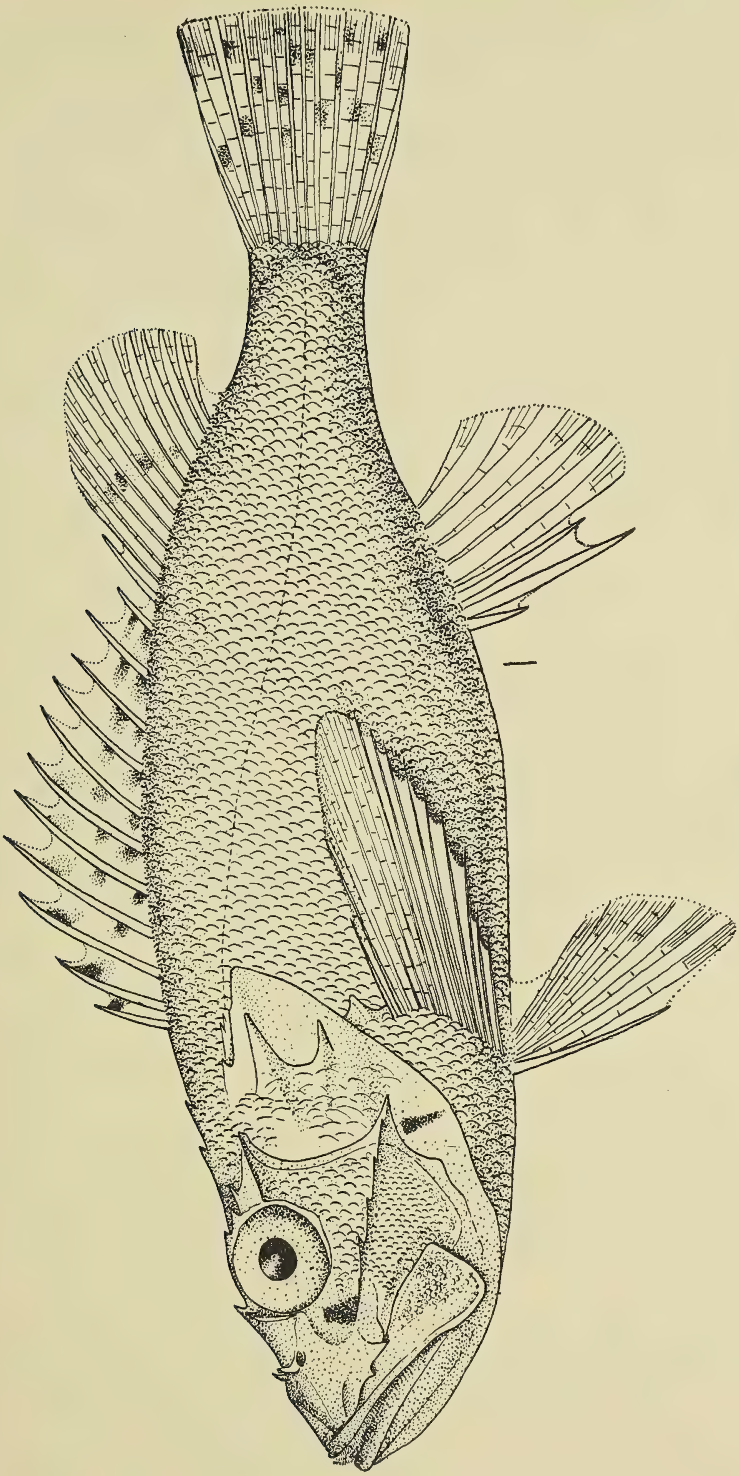

FIG. 13. *Neomerinthe tortugae* Hildebrand, n. sp. From the type, 245 mm. long.

Head in standard length 2.4; depth 3.7. Eye in head 5.15; snout 3.15; maxillary 2.1; interorbital 7.75; caudal peduncle 4.2; 3d dorsal spine 2.8; 2d anal spine 2.5; ventral 1.75; pectoral 1.65. D. XII, 11; A. III, 5; P. 17; scales 6-62-12; gill rakers 9.

Body elongate, moderately robust; head large; snout rather pointed; mouth large, slightly oblique; lower jaw included, its tip fitting into a deep notch in upper jaw; maxillary broad, extending fully to posterior margin of pupil. Teeth in jaws in bands, small, pointed, the bands in upper jaw widely separated anteriorly; similar teeth present also on vomer and palatines. Gill rakers consisting of low, spiny tubercles. Scales small, ctenoid, extending forward on interorbital and snout, and also covering upper part of expanded part of maxillary; those on top of head, snout, maxillary, and in part on cheeks very small. Spines on head moderately prominent, 1 pair over anterior nostrils, 4 on supraorbital ridge, and 2 more on nape, 3 behind eye; 3 spines on preopercular margin, upper one with a supplemental spine at base; 2 on opercle; 2 on preorbital, both directed downward and backward; and 4 on suborbital keel.

Dorsal fin deeply notched, spinous and soft parts connected by a very low membrane, 3d, 4th, and 5th spines of about equal length and about as high as longest soft rays, latter almost reaching base of caudal if deflexed; caudal with slightly convex margin; anal small, 2d spine enlarged, much longer and stronger than 3d and nearly as long as longest soft rays; ventrals pointed, failing to reach vent by diameter of eye; pectorals rather long, not broad or fan-shaped, the 2 upper rays simple, the next 6 divided, and the lower 9 simple, reaching beyond tips of ventrals but not to vent.

The color of the preserved specimen is pale, with scattered dark dots on the head, the upper parts of the body, and the dorsal, caudal, and pectoral fins; the spots on the interradial membranes of the dorsal and caudal are horizontally elongate; the anal and ventrals are pale, the former with indications of white spots.

The two paratypes are the basis for the following proportions and counts: Head 2.4, 2.9; depth 3.4, 4.2; eye 4.5, 5.2; snout 3.2, 3.2; maxillary 2.2, 2.1; interorbital 9.2, 8.8; caudal peduncle 4.4, 4.5; 3d dorsal spine 2.6, 3.0; 2d anal spine 2.2, 2.4; ventral 1.6, 1.75; pectoral 1.5, 1.6. D. XI, 11, XII, 11; A. III, 5, III, 5; P. 17, 17; scales 6-60-12, 7-65-12; gill rakers 9, 9.

The smaller paratype is rather less robust than the other two specimens, and the gill rakers are somewhat longer, indicating that they become shorter with age. Only 3 of the median pectoral rays show signs of branching distally, whereas 6 and 7 show branching respectively in the type and the other paratype. This apparently indicates either variation with age or possibly great variation among individuals.

The smaller paratype also is much more profusely spotted than the larger specimens, black spots being present on the anal fin as well as on the dorsal, caudal, and pectorals, though not on the ventrals, which are slightly dusky distally.

The color of the specimens when fresh is described in Dr. Longley's field data as "rosy, much speckled with brassy yellow, but at 145 mm. brown-spotted instead of brassy."

This species seems to be rather near *N. hemingwayi,* with which it agrees rather closely in color, as described and figured by Fowler (see citation above). However, its body is more slender, the head is smaller, the scales are notably more numerous in the lateral series, and the 2d anal spine is longer. Fowler gave the following proportions: Depth 3.0; head 1.12; 2d anal spine 3.12 in head. The scale count in the lateral series (just above the lateral line) he gave as 38, whereas the type of *tortugae* has 62. S. F. H.

Scorpaenodes floridae Hildebrand, n. sp.

Two specimens, respectively 48 and 58 mm. long, taken south of Tortugas in 45 fathoms, apparently are representatives of a new species. The smaller one, with standard length of 39 mm., is selected as the type, because it is in better condition than the larger one. The specimens are deposited in the U. S. National Museum, the type bearing catalogue number 108875 (fig. 14).

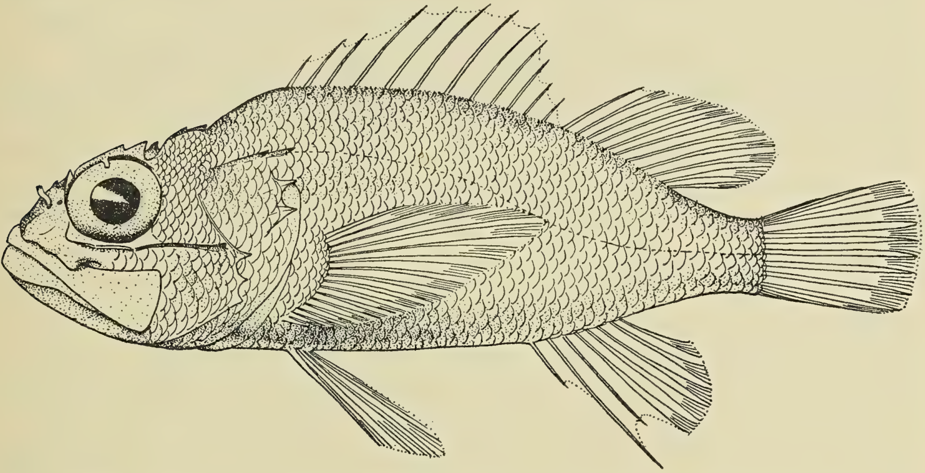

Fig. 14. *Scorpaenodes floridae* Hildebrand, n. sp. From the type, 48 mm. long.

These specimens proved bothersome to Dr. Longley, as I found a note stating, "Worked with two Scorpaenids a full half-day." Though he inserted no name in his notebook, the name "*Pontinus macrolepis* Goode and Bean, juveniles" is with the specimens. However, the presence of 13 dorsal spines and the absence of palatine teeth show that the specimens fall into the genus *Scorpaenodes,* of which apparently only four species previously had been found in the western Atlantic.

In the following description the proportions and counts of the type are given first, and if the paratype varies noticeably from the type that fact is stated.

Head in standard length 2.3, 2.3; depth 2.6, 2.9. Eye in head 3.1, 3.6; snout 4.25, 4.4; maxillary 1.75, 1.9; interorbital 7.4, 6.7; caudal peduncle 4.25, 4.4; 4th dorsal spine 3.3, 2.9; 2d anal spine 1.9, 1.8; pectoral 1.3, 1.3; ventral 1.4, 1.7. D. XIII, 9, XII-I, 10; A. III, 5, III, 5; P. 17, 17; scales 5-37-9, 5-38-9; gill rakers 9, and 2 rudiments.

Body considerably compressed; head moderately large, its greatest width about equal to its depth at middle of orbits; dorsal profile convex, except for a depression which is scarcely a pit at occiput; snout rather short, and broad; mouth large, oblique; lower jaw included; maxillary broad, reaching posterior margin of eye. Teeth in jaws villiform, in rather broad bands, the one on upper jaw interrupted anteriorly; vomer with a V-shaped patch of villiform teeth; none discernible on palatines. Gill rakers short, spiny, slightly expanded at tips, longest scarcely half diameter of pupil. Scales rather large, finely ctenoid, extending forward on head slightly beyond posterior margin of eye; large scales also present on cheeks and opercles. Spines on head rather prominent; 1 pair on snout between nostrils; 1 pair on anterior part of high supraorbital ridges, and 2 pairs on these ridges posteriorly; 1 pair behind eyes; 2 pairs of prominent ones along sides of occipital depression; 2 spines at origin of lateral line, the 2d one prominent; 2 spines on opercle; 1 halfway between base of pectoral and lateral line; 3 on preopercular margin, upper and largest one with a supplemental spine at base; no definite preorbital spines; suborbital keel in three parts, with a terminal spine posteriorly only; a simple dermal flap, nearly as long as pupil, with origin between bases of the 2 spines over posterior part of supraorbital ridge; a short and broader dermal flap attached to upper anterior part of eye; no dermal flaps evident on body.

Dorsal fin deeply notched, the two parts connected by a low membrane between 12th and 13th spines, first 3 spines graduated, but much shorter than the 4 or 5 succeeding ones, which are of nearly equal length, succeeded by much shorter ones; soft part of fin notably shorter than spinous part, longest rays about as long as longest spines, reaching somewhat beyond base of caudal if deflexed; caudal with convex margin; anal small, 2d spine much longer and stronger than 3d, but scarcely as long as longest soft rays, which reach base of caudal if deflexed; ventral reaching vent, spine strong; pectoral rather long, reaching a little beyond origin of anal, 2 uppermost rays simple, next 4 divided, and lowermost 11 simple in the type, the division in the paratype being 1 + 7 + 9 (probably varying somewhat with age).

The fresh specimens were described in Dr. Longley's field data only as "redfish." They have faded in preservative and are now straw color, the only color markings remaining being a rather large blackish spot on the posterior part of the spinous dorsal, and some groups of dark chromatophores in the axil of the pectoral, these markings being more distinct on the paratype.

This species differs from the other species of the genus known from the Atlantic coast of America in having only one spine on the suborbital keel, which is on the terminal end. It differs from most of the American species also in having fewer pectoral rays, a larger mouth, and fewer scales in the lateral series.

S. F. H.

Peristedion spiniger Longley and Hildebrand, n. sp.

In addition to four known species of *Peristedion,* all well marked, a fifth one occurs in the collection for which no name or description has been found. There are two definite records in Dr. Longley's field notes of the capture of this species, identifiable by tin tags. One lot originally consisting of 9 specimens, 120 to 175 mm. long, was taken July 19, 1932, in 295 to 315 fathoms, and the second lot, consisting of 11 specimens, 112 to 160 mm. long, was taken August 1, 1932, in 197 fathoms. However, it is evident that Dr. Longley had specimens as early as 1931, because he made "Notes on *Peristedion*" for that year in which he listed "*Peristedion* sp." sufficiently described to identify his material with the present species. It is not stated where the specimens were taken.

A specimen 183 mm. long (standard length, measured without rostral processes, 150 mm.), taken in 295 to 315 fathoms, has been selected as type. It is deposited in the U. S. National Museum under catalogue number 108866 (fig. 15).

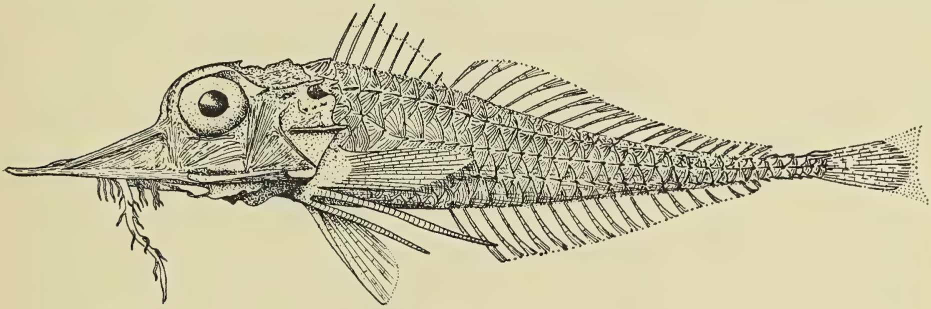

Fig. 15. *Peristedion spiniger* Longley and Hildebrand, n. sp. From the type, 183 mm. long.

In the following description the proportions and counts based on the type are enclosed in parentheses. The other proportions and counts are based on 4 paratypes having a total length of 135 to 170 mm.

Head in standard length (both measured without rostral processes) 2.6 to 2.75 (2.6); depth 6.1 to 6.3 (5.9); anal base 2.4 to 2.5 (2.4). Eye in head (measured without rostral processes) 4.1 to 4.6 (4.4); snout (without processes) 2.25 to 2.4 (2.3); interorbital 4.2 to 4.7 (4.75); width of head 1.4 to 1.5 (1.4); rostral process 4.4 to 4.9 (5.0); ventral 1.75 to 2.2 (2.2); pectoral 1.9 to 2.1 (2.1). D. VIII - 18 or 19 (VIII - 18); A. 18 to 20 (18); P. 12 + 2 (11 + 2); scutes in lateral line series 4 + 28 or 29 + 1 (4 + 28 + 1); gill rakers 21 or 22 (22).

Head and body depressed, broader than deep throughout length; head broad, though not broader than in *P. miniatum* and *P. platycephalum;* rostral processes moderately long and broad, about equal to diameter of eye. Spines on head not prominent; supraorbital ridge with no spine in front and only one small one posteriorly; ridge of preopercle expanded, without spines; spines on body rather prominent and sharp, those in arch of lateral line series being more strongly developed than in the other local species; last spine in lateral line series on base of caudal not reduced, sharp and serrate; 9 to 11 of scutes preceding last 2 in lateral line series with 2 spines, anterior one directed forward; only one of the numerous barbels on lower jaw enlarged, reaching nearly or quite to base of ventral if laid back, nearly as long as eye and snout without process, scarcely as long and not as thick as in *miniatum,* though longer than in the other local species. Origin of first dorsal about over base of pectoral, narrowly separated from long, low second dorsal; caudal slightly concave; anal similar to second dorsal; ventral fins inserted under base of lower free rays of pectorals, reaching slightly beyond vent, adherent to abdomen by a membrane for about two-thirds their length; pectorals rather long, reaching to or even a little beyond origin of anal, the upper free ray being the longest in the fin.

I have found no notes on the color of fresh specimens. The preserved ones are pale straw color; lower surface of head, chest, and abdomen white; no silvery band on side. The top of the head, particularly the snout, has short brownish streaks, the most prominent one being between the bases of the rostral processes, forming a broad angle with the apex on the median point of the margin of the snout. Several brownish spots follow the curve of the lateral line anteriorly. The first dorsal is largely black; the second dorsal at most with some dusky spots; the caudal fin frequently with dusky markings; the anal and ventral fins plain; the pectoral fins pale, with only a few dusky spots in some specimens, much of the fin dusky in others.

This species is related to *miniatum* and *platycephalum*. It differs from both in the more strongly developed spines on the scutes in the arched part of the lateral line. It differs prominently from *miniatum* in the fewer and smaller spines on the head, the present species having only a single small spine on the supraorbital ridge, which is placed posteriorly, whereas *miniatum* has 3 principal ones, with auxiliary ones, over each ridge. The rostral processes are much longer in *spiniger,* and the largest mandibular barbel is rather shorter and more slender. From *platycephalum* the present species differs in having a smaller eye and a much longer mandibular barbel, and in the absence of silvery coloring on the lower part of the side. S. F. H.

Prionotus microlepis Longley and Hildebrand, n. sp.

The Tortugas collection contains 26 specimens, 70 to 133 mm. long, which Dr. Longley apparently failed to identify with any known species, and I too have failed. One of these specimens was taken west of White Shoal in a haul with the otter trawl in water ranging in depth from 7½ to 11 fathoms. No definite locality data are furnished with the other specimens. However, "*Prionotus* sp." is listed several times from the channels in the keys. It apparently may be assumed that the other specimens are included in the designation mentioned, and that they perhaps were all taken in the local channels.

These specimens perhaps are nearer *P. ophryas* than any other known species; the scale, gill-raker, and fin-ray counts agree almost completely with that species. The present specimens disagree, however, in having a definite groove across the top of the head behind the eyes. Furthermore, the opercular and preopercular

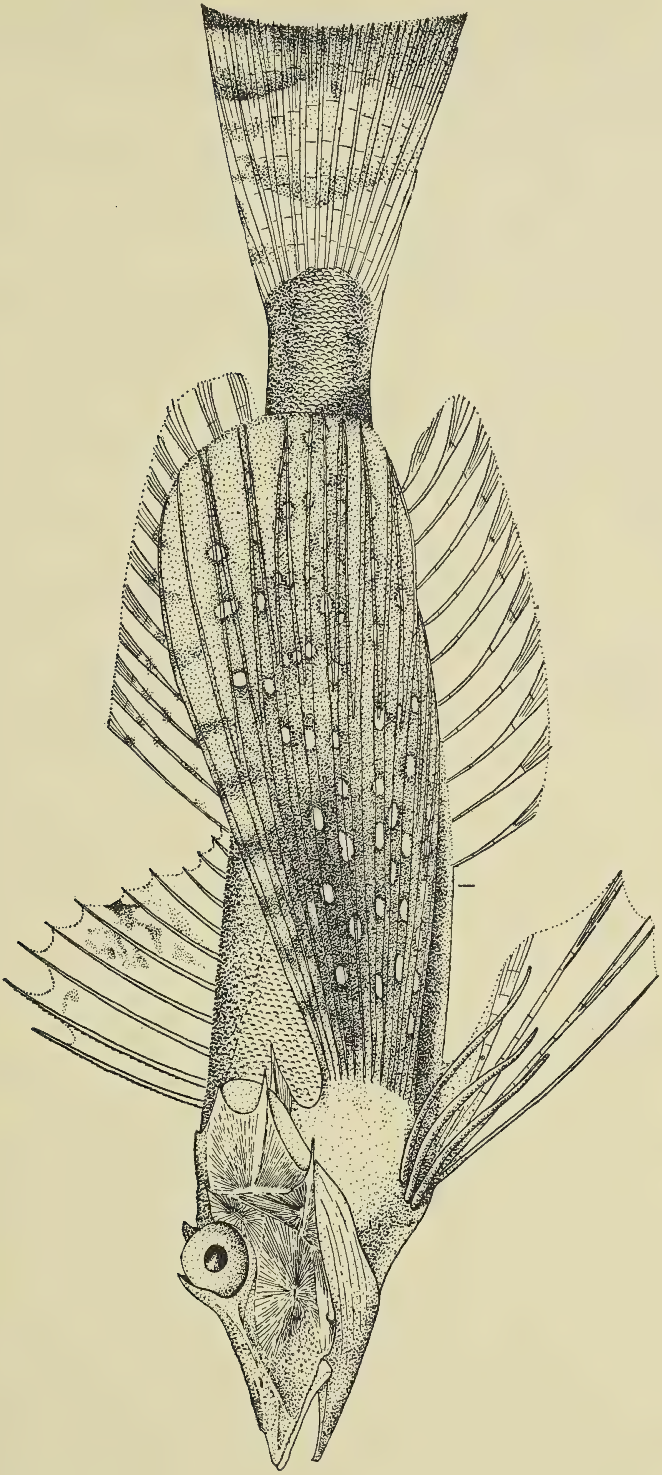

Fig. 16. *Prionotus microlepis* Longley and Hildebrand, n. sp. From the type, 133 mm. long.

spines are longer, both reaching beyond the membranous opercular margin, and no nasal or ocular barbels are present. Though the pectoral fins are long, they are not so long as in *ophryas,* as they generally do not quite reach the end of the base of the second dorsal.

From *P. alatus,* with which the counts usually made also agree rather closely, the present species differs both in the absence of a greatly produced ray in the pectoral, and in the absence of a recurved spine at the angle of the snout, the margins being serrate, without enlarged spines.

The specimen with definite locality data, which is 133 mm. long (standard length 102 mm.), is designated as type, and is deposited in the U. S. National Museum as catalogue number 108869 (fig. 16). In the following description the proportions and counts enclosed in parentheses are based on the type, and the others on 6 paratypes, ranging in length from 73 to 135 mm.

Head in standard length 2.6 to 3.1 (2.8); depth 4.5 to 4.9 (4.9); ventral 3.0 to 3.2 (3.1); pectoral 1.5 to 1.7 (1.6). Eye in head 4.7 to 5.3 (5.3); snout 2.0 to 2.25 (2.2); interorbital 11 to 14 (12); maxillary 2.9 to 3.4 (3.3); mandible 2.5 to 2.8 (2.8); third dorsal spine 1.6 to 2.0 (1.9). D. X - 12 (X - 12); A. 11 (11); P. 13 + 3 (12 + 3); scales 92 to 100 (92); pores 50 to 52 (52); gill rakers 7 to 9 (7) and some rudiments on lower limb of first arch.

Body rather slender; profile over snout nearly straight; head moderately small, scarcely broader than deep; supraorbital ridges moderately high; interorbital narrow and deeply concave; a definite groove across head behind eyes. Spines moderate; margins of snout serrate, without a prominently enlarged spine; no spine at center of striations on cheek; preorbital serrate, with last tooth over anterior part of eye slightly enlarged; a few low spines on orbital ridge just in advance of occipital groove, and a few low serrate ridges behind it; humeral spine rather flat, serrate on outer margin; upper opercular spine obsolete, 2d rather long, reaching well beyond membranous margin of opercle; preopercular spine long, reaching well beyond margin of opercle, no supplementary spine at its base; no cirri on head. Gill rakers few and rather slender, scarcely as long as pupil. Scales small, ctenoid, reduced and cycloid on chest; first 3 or 4 scales in lateral line modified, more like scutes. Dorsal spines moderately high, 1st strongly serrate along its entire anterior border, and next 2 at least in part with serrae, 2d and 3d spines of about equal length, and fully half the diameter of eye longer than 1st; second dorsal long and low; caudal with slightly concave margin, lower rays slightly longer than upper; anal similar to second dorsal, its origin directly under that of second dorsal; ventrals rather pointed, longest ray reaching somewhat beyond origin of anal; pectorals long, generally but not always failing to reach end of base of dorsal, distal margin convex.

I am unable to find notes on the color of fresh specimens definitely identifiable with this species. The ground color of preserved specimens is grayish brown above, pale underneath. Body frequently with dusky blotches, especially along lower part of side, and generally with brown freckles on upper parts (these missing in the type). Dorsal and caudal fins pale, with dark spots, these often arranged to form bars on the caudal, the margin of which is always dusky; the spinous dorsal with one and often with two dark spots on the margin of the membranes between the 4th and 6th spines. Anal and ventrals pale; pectoral largely brown to black, with alternating dark and pale bars on upper part, and the rest of fin uniform brownish with bluish spots. S. F. H.

Microgobius microlepis Longley and Hildebrand, n. sp.

Compressed, the male more slender than the female, depth 4.25 and 4.0 in representative specimens of the two; head 3.5 and 3.2 similarly; eye 4.4.

Fin formulae usually D. VIII, 17½; A. I, 18½. In 12 males and as many females examined, 15½, 16½, and 18½ dorsal rays were present each in one fish, and 2 had 17½ in the anal. In 12 specimens of *Microgobius signatus* (U.S.N.M. No. 35164) collected at Havana the fin formulae with exceptions noted are D. VIII, 19½; A. I, 20½. One has the dorsal rays 18½, one 20½; two have the anal rays 19½, and one each 20 and 21. The indicated difference of 2 rays in dorsal and anal fins is the most obvious of the marks which distinguish these species.

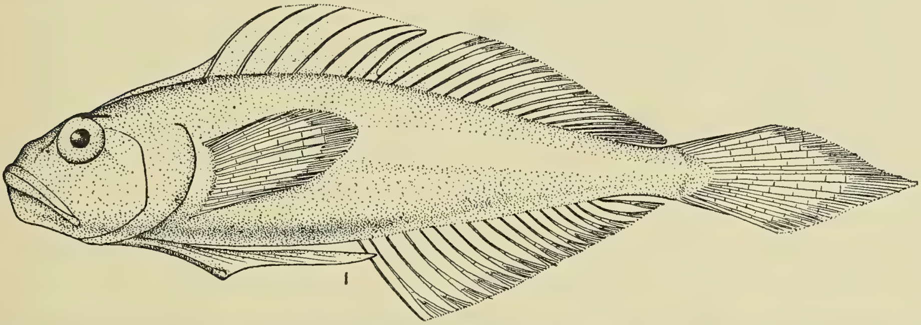

FIG. 17. *Microgobius microlepis* Longley and Hildebrand, n. sp. From the type, 53 mm. long.

Anterior profile not decurved or steep. Mouth large, very oblique; maxillary reaching a point below pupil. Teeth all incurved canines; outer series in upper jaw anterior or anterolateral, about 6 on either side, enlarged and irregularly spaced; an inner series single, very much more numerous than the first, regularly spaced, varying little in size, the smallest in the mouth; teeth in lower jaw in a single series except anteriorly, where there are 1 or sometimes 2 rows outside the main series; outer ones may be more or less distinctly enlarged; posterior 2 or 3 teeth in outer series are largest and may equal any in upper jaw. Head, nape, and breast naked, as well as a narrow strip along base of first dorsal fin anteriorly. Scales chiefly cycloid, weakly ctenoid behind pectoral fin, small anteriorly and very difficult to count, about 85 in series from axil to base of caudal fin. Bones of skull thin with a median ridge rising in interorbital space in transverse plane of posterior margins of orbits; this surmounted by a compressed fleshy crest extending from interorbital space to origin of dorsal fin, with which it is continuous. Double series of papillae along mandible below everted lip; another series along anterior margin of preorbital; horizontal series across cheek. The two dorsal fins contiguous, spines filiform at tip, not exserted, 5th and 6th longest, not extending beyond base of 3d ray; last rays of dorsal and anal extending when depressed to the reduced rays at base of caudal. Ventrals longer than pectoral, longer in male than in female, reaching in the former in mature fish to or beyond origin of anal fin, in the latter scarcely to vent.

The species is sexually mature at or slightly below the length of 50 mm., and is dimorphic in coloration. The ground color is very faint pearl gray in both sexes, or sometimes palest of gray with a slight olivaceous tinge. The flesh is translucent. From the eye to the premaxillary margin before the rictus, and extending backward on the cheek from near the rictus, are microscopic lines of yellow, between which are several irregular iridescent bluish spots with another following upon the opercle.

The first dorsal fin of the male is very pale orange over its basal two-thirds, and for the rest bluish. The basal two-thirds of the second dorsal is a somewhat brighter orange, the distal third colorless with a submarginal line of bright yellow of about one-quarter the width of the pupil. In almost perfect alignment with this yellow line a similar streak of yellow runs from the dorsal margin of the caudal base straight to the tip of the fin. The pectoral is colorless and the anal and ventral fins straw color. In the female the first dorsal, a very small area at the base of the first three or four supports of the second dorsal, and the ventrals are slightly pigmented, the remaining fins being colorless. On the body of the female, however, there is a very distinct mark in the form of a colorless area bounded before by an arc convex posteriorly and extending from the base of the 4th dorsal ray to the anal spine. From the same point beneath the dorsal fin a second arc, convex ventrally, descends to the mid-level of the side and thence to the lower base of the caudal.

Neither sex shows such a mark above the base of the pectoral as is shown in Metzelaar's figure of *Xenogobius weberi,* or as is said to occur in *Microgobius signatus,* and does appear in females in the lot of fishes from Havana. Female *signatus* will probably show upon the side a mark homologous with that described above, although no more than a trace of it remains in the faded alcoholic material.

Microgobius microlepis is common over parts of the soft, muddy bottom of the northern deep holes in the Bird Key flats. Twenty-eight specimens 30 to 55 mm. in length were taken with dynamite within a radius of 3 meters. In the breeding season at least, the fish live in burrows, which are apparently excavated by the males alone. I have often seen them at work energetically. They carry out much sand in their mouths, and they also go into their holes and by strong swimming movements drive out a continuous jet of fine sand. The females, too, sometimes go into the holes but bring out nothing. While the males are working they sometimes move a short distance away and here and there daintily pick something to eat from the bottom. In the stomachs of a few examined, copepods, ostracods, and a few foraminifera were found.

Breeding continues throughout the summer. Mated males and females then share one burrow, above which they may be found "floating" together and into which they dart, head foremost, when alarmed. The unmated live round about, but I noticed among them no female in full breeding condition. W. H. L.

Although Dr. Longley recognized this goby as new and prepared the foregoing description, he did not name it, nor did he select a type. These duties, therefore, fall to me. I have chosen the name *microlepis,* in reference to the very small scales, which cannot be enumerated accurately.

The type selected is a male having a total length of 53 mm., and standard length of 48 mm. This specimen is registered in the U. S. National Museum under catalogue number 108868 (fig. 17). The following proportions and counts are based on the type: Head in standard length 4.4; depth 6.0. Eye in head 3.7; snout 5.4; interorbital 10; maxillary 2.0; pectoral 1.35. D. VIII, 17; A. I, 17.

The preserved specimens have faded and are now virtually colorless, except for the two arcs described in fresh material, which remain and are slightly dusky, the one between the origin of the anal and the 4th ray of the second dorsal being more distinct than the other.

The collection contains, in addition to the type, 35 paratypes ranging in length from 27 to 52 mm. S. F. H.

Opisthognathus fasciatum Longley, n. sp.

This fish comes from gravelly bottom, sometimes from near shore below the low of neap tides, and sometimes with *O. whitehurstii* from mixed sand and gravel bottom such as is found north of Loggerhead Key. It is a burrower and builder like the other members of its genus. During the day it holds fast to its shelter, as *O. maxillosum* and *O. whitehurstii* do, and it does not swim and feed above it like *O. aurifrons.* It may be caught easily by inverting a jar over it and pushing a flat file or stiff wire rod through the shaft of its burrow below it. A little shaking will then tumble the wall down and drive the fish up out of the wreckage. The largest ones seen are about 75 mm. long. None taken were sexually mature, so the species may grow at least a little larger.

In 10 specimens closely examined, 8 had the dorsal fin formula XI, 16½, and 2 one ray less; in the same 10 fish, 3 had A. III, 15½ and 7 had III, 16½; pectoral rays 19 to 21; scales in lateral series about 90. The pores of the lateral line system, except for a very short distance at the anterior end, are in single series with an accessory pore above and below at regular intervals. Scales above the lateral line begin in a single row under the 9th spine and increase to 3 or 4 rows at the close. Teeth rather strong, wide-set, in two series, the inner series limited to a few teeth anteriorly, and these directed backward, particularly in upper jaw; vomer with a single strong tooth. Maxillary with a supplemental bone, a mere sliver, long, narrow, slightly arcuate, pointed at both ends, ending far in advance of maxillary tip, widest at its anterior point of trisection, its greatest width between one-quarter and one-fifth its greatest length. Maxillary itself truncate or very slightly notched posteriorly and extending half the distance from vertical of posterior margin of orbit to preopercular margin. Gill rakers 12 above, 23 below angle of anterior arch, long, slender, compressed, with a few teeth on basal three-fifths of pharyngeal margin, the distal ones widely spaced. Eye large, 3.0 in head; snout short, twice the very narrow interorbital space, and less than diameter of pupil.

Upon a very indistinctly mottled or marbled ground this species shows, more diagrammatically than others, a pattern common to three of them. A dark bar at the anterior margin of the interorbital space extends across the fore part of the eye and thence horizontally across the iris, including the pupil. Upon the eye it is sharply set off above by the pale ground color upon the conjunctiva, while the same color upon the iris defines it below. Two other dark bars, a postorbital and a nuchal one, are sharply drawn on the dorsal surface, and extend with some irregularities upon the cheek and operculum. An anterior extension of the postorbital bar extends forward into the interorbital space.

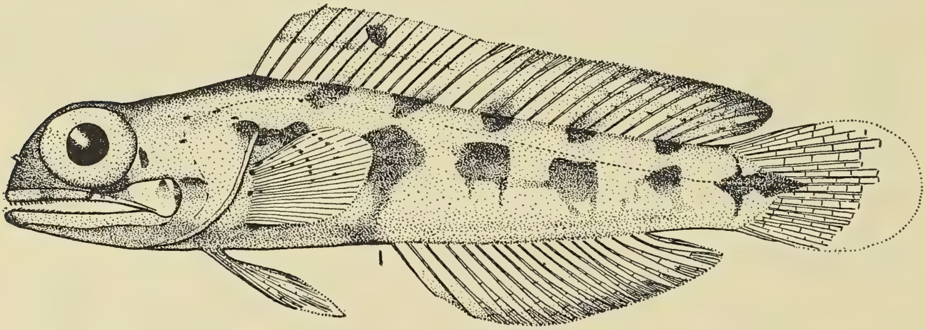

Fig. 18. *Opisthognathus fasciatum* Longley, n. sp. From the type, 64 mm. long.

At the base of the dorsal fin is a series of seven or eight dark blotches. The first is narrower than those following, and lies under spines 2 and 3. It extends ventrally upon the tip of the opercular flap. The second is under spines 4 to 6, the third under spines 8 to 10, and the others follow at distances only slightly diminished upon the tapering caudal peduncle. At mid-level upon the side is a corresponding series of darker brown markings.

With changes in the shade of the fish as a whole, and minor changes in the extent of the spots themselves, these blend to form a very definite brown line from pectoral to caudal, or, by extension in the other direction, unite with their fellows above and stretch ventrally as well to form clear-cut brown bars on the pale ground color. The pectorals and ventrals are finely spotted with dark on the rays. The dorsal is pale at the base and otherwise in general pale olivaceous, except for a second pale stripe which runs concurrently with the margin from the tip of the last spine to the middle of the last ray and cuts off a marginal band of the olive as wide as itself. High up on the fin from the 7th to the 9th spine is a dark spot homologous with a much more extensive semi-ocellated one in *O. maxillosum*. The anal is olivaceous, darker toward the border. The caudal is the same, with a longitudinal dark streak often plainly showing through its middle, and with faint white spots besides.

Ostracods, copepods, amphipods, isopods, and foraminifera were included in the stomach contents. W. H. L.

Dr. Longley named and described this species in his manuscript, as shown above, but he apparently did not designate a type. Therefore, the duty of selecting one falls to me. I have chosen from among 13 fish, ranging in length from 30 to 72 mm., a specimen 64 mm. long, standard length 53 mm., which is number 108873 in the U. S. National Museum collection (fig. 18).

The following proportions and counts are based on the type: Head, measured to end of opercular spine, 3.1 in standard length; depth 4.7. Eye in head 2.55; snout 8.2; interorbital very narrow, about 17; maxillary 1.4; caudal peduncle 3.4; ventral 1.55; pectoral 1.9. D. XI, 16; A. II, 16; P. 20; scales too small to enumerate accurately, about 110, none above lateral line anterior to base of 7th dorsal spine, where one partly embedded row begins, increasing to about 4 rows under base of 5th soft ray, where lateral line ends; gill rakers 21 in a specimen not the type.

The type, which does not differ greatly from the other 12 specimens at hand, in preservative is brownish. It has six dark blotches on the back under the base of the dorsal, which do not extend on the base of the fin, the first one (under the anterior spines) quite indistinct. There are seven dark blotches along the middle of the side, which are separate and do not form a continuous lateral band. Each blotch has an indefinite dorsal and ventral extension, but the dorsal extensions scarcely reach the blotches on the back, and therefore scarcely form crossbars. An additional dark spot is present on the base of the caudal, with a forward and a backward extension, connecting it with the spot in advance of it and reaching beyond mid-length on the caudal rays. A pair of pale spots lies on the base of the caudal, occupying the base of the fin above and below the interspace between the last spot in the lateral series and the one on the caudal fin. The head retains no dark bands or other markings, exclusive of some dark points behind the eye, on the maxillary, and on the gill covers, which occur also along the lateral line. The dorsal fin is brownish, with a pale band at the base, a pale margin on the spinous part, and an intramarginal pale band on the soft part. A rather prominent black spot lies high up on the dorsal, between spines 7 and 9. The ventral and anal fins are finely punctuated with brown, the anal darkest along its margin; pectoral translucent with definite dark dots like those on the head.

The color, and the peculiar supplemental maxillary bone, characterize this species. S. F. H.

Opisthognathus whitehurstii (Longley)

Gnathypops maxillosa Jordan and Evermann, Bull. U. S. Nat. Mus., vol. 47, pt. 3, 1898, p. 2284, fig. 801 (not of Poey).

Gnathypops whitehurstii Longley, Carnegie Inst. Wash. Year Book No. 30, 1931, p. 395—Tortugas, Florida. (Notes, but no description.)

Probably locally the commonest of the Opisthognathidae. Sometimes it is found on the coarse gravelly bottom that harbors *O. aurifrons,* but more often it was discovered among coral fragments along the raw edges of *Thalassia* fields where erosion was in progress. It lives in burrows excavated by its capacious jaws, armed though they are with only weak recurved canines.

An individual exposed by destruction of its chamber was pursued by *Halichoeres bivittatus,* which it narrowly escaped. After having been driven up several feet in the open water it came down near a bit of dead coral. Removing a dozen mouthfuls of sand in as many seconds, it was comfortably ensconced again. Much, doubtless, was done afterward to make the refuge safer, but when all was completed it would remain merely a hole in the ground, since this species does no mason work comparable to that of *O. aurifrons* and *O. maxillosum.*

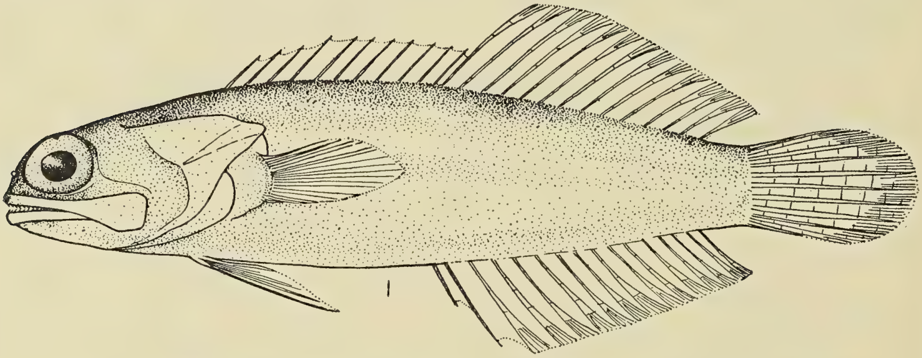

Fig. 19. *Opisthognathus whitehurstii* (Longley). From the type, 68 mm. long.

This is strictly a bottom species, never "floating" above its burrow, nor seeming to leave it more than momentarily by day, except under compulsion. When *Halichoeres* passes by it cowers in its fastness, but holds its ground with gaping, defiant mouth if the intruder is no more than the harmless *Blennius favosus.*

This species has been represented in the U. S. National Museum collection (No. 5689) in Washington for many years by a specimen collected at Tortugas by Dr. D. D. Whitehurst, in commemoration of whose service it is named. Having been mistaken for *Gnathypops maxillosa* Poey, these specimens serve as the basis for the description of that species by Jordan and Evermann (see reference above).

As the fin formulae of this species Poey gave D. VIII, 18; A. II, 15. For their species Jordan and Evermann's text gave D. VIII, 17; A. II, 13. Their accompanying figure showed D. XI, 15; A. 16, which is within the normal range of variation. In 16 specimens taken at random I obtained this distribution of fin supports: D. X, 15 in 1, XI, 15 in 10, XI, 16 in 4, XII, 15 in 1; A. II, 14 in 1, II, 15 in 2, III, 14 in 10, and III, 15 in 3.

Pores in lateral line system in double series terminating under 1st soft ray of dorsal; only 1 row of scales between it and dorsal base, except anteriorly, where there are 2; these scales begin under 4th spine. Teeth conical, slightly recurved, not widely spaced; in upper jaw in a single row; lower jaw with a complete outer series and with 3 or 4 only in an inner series anteriorly; teeth on vomer usually 2, but sometimes wanting. Maxillary truncate, extending halfway from vertical of orbital margin to posterior margin of preopercle. Gill rakers long, slender, compressed, with few teeth widely spaced on basal half of pharyngeal border.

Gray and olive are its colors. Its dark blotches fall in seven transverse bands, darkest at mid-level on the side and so tending to give the effect of a dark lateral stripe. In the larger fish a hazy dark spot on the anterior part of the dorsal web corresponds to a well-defined blue one in the smaller examples. There are two white or colorless spots at the base of the caudal. In shade the fish is changeable. On bare sand the body above the level of the eyes may be gray mottled, with seven bands only faintly showing. W. H. L.

I have made some measurements and counts of the specimen in the National Museum (No. 5689; fig. 19), which became the type when Dr. Longley discovered that it was not *O. maxillosum* Poey and named it *Gnathypops whitehurstii* (see references to Jordan and Evermann and to Longley above). The results of these measurements, together with others based on a specimen in the Tortugas collection, are as follows, the figures in parentheses pertaining to the last-mentioned fish: Total length 68 (40) mm.; standard length 56 (30) mm. Head, measured to tip of opercular spine, 3.1 (2.5) in standard length; depth 4.3 (3.75). Eye in head 3.7 (3.1); snout 7.2 (9.2); interorbital about 25 (about 35); maxillary 1.65 (1.7); caudal peduncle 3.5 (3.6); pectoral 1.5 (1.7). D. XI, 14 (XI, 14); A. II, 14 (III, 13); P. 17 (18); scales about 55 (54); gill rakers 18 (19).

The proportions and counts based on the type agree fairly well with those given by Jordan and Evermann (if the total number of dorsal rays is considered), except for the proportion of the head. If, however, the total length of the fish is divided by the length of the head, Jordan and Evermann's proportion is obtained. The scales are not easily counted, and much depends upon where the count is begun anteriorly, as there are scale marks in advance of scales having free margins. In my counts scales having more or less free margins only are included.

The color of the type has faded to a uniform plain brownish gray, the head and lower parts being lighter than the rest of the body, and the fins are all plain translucent.

The Tortugas collection contains 22 specimens, ranging in length from 23 to

53 mm. These specimens differ markedly among themselves in shade, some being much darker than others, the ground color varying from a rather deep brown to almost gray. The dark blotches described by Dr. Longley remain essentially as described by him, and are most distinct on the lightest-colored individuals. In addition to a blackish spot on the anterior part of the dorsal fin, extending from the 2d to the 4th or 5th spine, the fin is punctulate with brown, and also has larger brownish spots. There is also an indication of a pale longitudinal band at the base, and another one on the soft part. The caudal is spotted with brown, forming irregular crossbars in some specimens. On the base of this fin, above and below the lateral line, the pair of rather large pale spots mentioned in fresh material by Dr. Longley remain distinct. The anal is spotted similarly to the dorsal; the ventrals are uniformly brownish; and the pectorals are plain translucent.

The markings in this fish, though somewhat similar to those in *O. maxillosum* and *fasciatum,* are finer, the dorsal and lateral spots being decidedly smaller, and in most specimens less distinct. The position of the dark spot on the dorsal, which is between the 2d and the 4th or 5th spines, is distinctive, as it is farther back, that is, between about the 7th and the 10th spines, than in the other two species mentioned. S. F. H.

Benthoscopus Longley and Hildebrand, n. gen.

The specimen on which this genus is based agrees with the Old World genera *Uranoscopus* and *Pleuroscopus* and the New World genus *Astroscopus* in having most of the body covered with scales, but it differs from them in the absence of a spinous dorsal. In the absence of the first dorsal it agrees with the other two genera represented in the local fauna, namely *Kathetostoma* and *Execestides.* From these it differs, however, in having most of the body scaled instead of entirely naked. It is characterized, furthermore, by the peculiar development of the dentary bones, the free margins of which do not unite anteriorly to form an arch as usual, but remain separate and form two prominent parallel bony ridges on the anterior part of the lower jaw close to the median line (see fig. 21).

S. F. H.

Benthoscopus laticeps Longley and Hildebrand, n. sp.

Two specimens,[2] the largest 285 mm. (standard length 205 mm.), were taken in two hauls, between depths of 90 and 175 fathoms.

Head, measured to margin of upper jaw, 3.0 in standard length; depth about 4.4; base of anal 3.8; base of dorsal 3.3. Eye in head 5.75; snout, measured to margin of upper jaw, 3.1; interorbital 3.8; maxillary 2.5; width of head 1.2; caudal peduncle 3.3; pectoral 1.4; ventral 2.9. D. 12½; A. 16½; P. 22; V. 6; scales along lateral line about 150.

Head and trunk broad and depressed, and tail compressed; head flat above, not concave, except for naked area over snout and between eyes; mouth large,

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[2] The second specimen has been lost. Accordingly the description is based wholly on the one specimen, the type, for which the length is stated (fig. 20).—S. F. H.

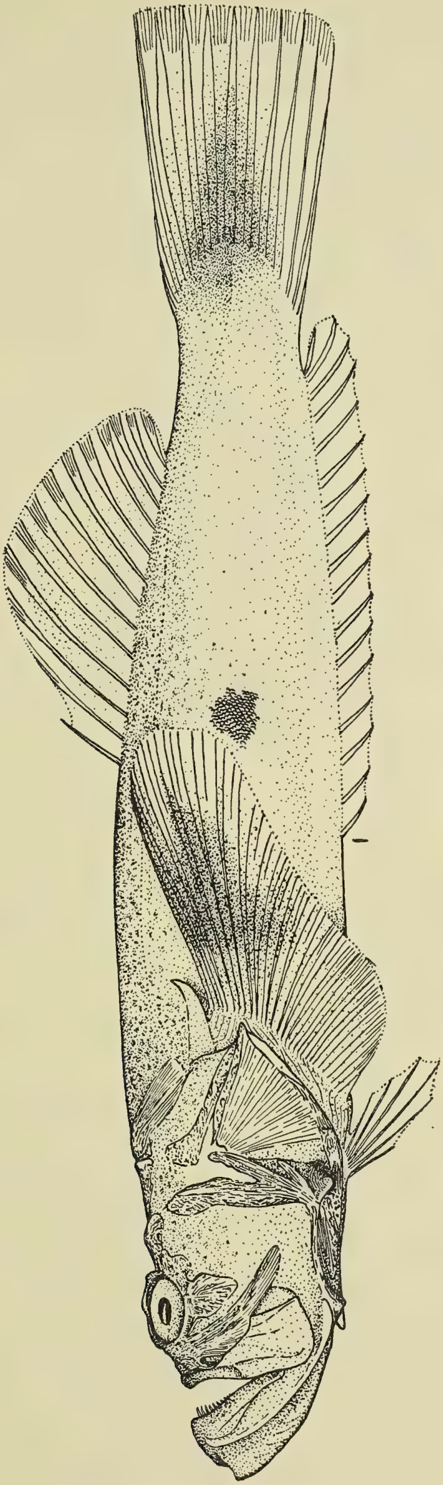

Fig. 20. *Benthoscopus laticeps* Longley and Hildebrand, n. gen. and n. sp. From the type, 285 mm. long.

vertical; preorbital with 4 flat ridges, upper 3 reaching border, but scarcely ending in spines; parietal with a low bony shield, with prominent radiating striae; preopercle with a strong, blunt, flattened spine projecting laterally at its angle a distance scarcely equal to half diameter of eye, this spine with 3 blunt ridges above; opercle with a sharp spine, terminating a low granulated ridge, descending obliquely along its upper border, and with a second ridge along lower border of opercle, leaving a triangular area between the ridges upon which striae radiate from upper anterior angle; humeral spine strong, curved upward, free part about as long as eye. Margins of dentary bones raised anteriorly, and remaining separate, forming 2 prominent parallel ridges at chin (see fig. 21).

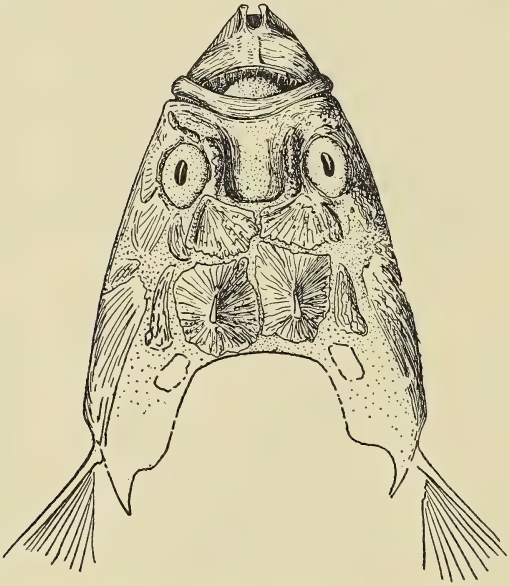

Fig. 21. *Benthoscopus laticeps* Longley and Hildebrand, n. gen. and n. sp. Upper surface of head, showing peculiar sculpturing and development of the dentary bones.

Teeth strong, in two series in upper jaw; in a single series in lower jaw; upper jaw with a pair of canines, one on each side of a median bare space. Pseudobranchiae well developed. Gill rakers short, blunt, spinose, 7 above angle and 14 below it on first arch. Scales at nape few and widely spaced; crowded along side, some embedded, cycloid, none on chest and abdomen. No tentacles about jaws; roof of mouth and tongue with minute ones. Lateral line runs upward toward posterior end of dorsal, with base of which it is parallel a short distance, and then is deflected sharply to middle of base of caudal.

Lower rays of pectoral much shorter than upper ones, longest ones reaching about opposite base of 5th ray of anal; ventrals inserted below margin of preopercle, short, reaching less than half the distance to vent; dorsal much higher than anal, its rays about 1.5 times as long as those of anal; caudal margin slightly convex, upper angle scarcely rounded.

Dorsal surface gray, with very many fine spots and dashes of olive, scattered at random. Sides and belly ashy below a line from humeral spine to middle line of base of caudal. Basal half to two-thirds of pectoral olive, the remainder rosy. Conjunctiva spotted like the back.

The color in alcohol is grayish brown above, paler below. Head uniform, without markings; back retaining spots and dashes, which have become dusky, not extending below the mid-line of the sides, where the body becomes paler, and is marked only with brownish punctulations. Dorsal grayish, becoming paler toward the margin; caudal dusky on basal two-thirds, then paler; anal and ventrals pale; pectorals dusky on basal two-thirds, outer third paler.

The sculpturing of the head in this species is much coarser than in *Kathetostoma albigutta,* though apparently no coarser than in *Execestides egregius.* It differs from both, as pointed out elsewhere, in being largely covered with scales. The number of fin rays in this species is about the same as in *E. egregius,* the dorsal having fewer rays than the anal, whereas in *K. albigutta* the anal has fewer rays than the dorsal, the numbers being almost exactly reversed.

W. H. L.

Dr. Longley had recognized the specimen upon which the foregoing description is based as unusual. He had questioned whether it belonged to *Kathetostoma* or to *Execestides,* neither of which it seemed to fit. However, he had come to no decision, and had suggested no new name or names, which were supplied by me. The preliminary description prepared by Dr. Longley was carefully checked and amended and enlarged.

The type is deposited in the U. S. National Museum under catalogue number 108879 (fig. 20). S. F. H.

Gillellus rubrocinctus Longley

Gillellus rubrocinctus Longley, Carnegie Inst. Wash. Year Book No. 33, 1934, p. 257—Tortugas, Florida.

The species is described from the type specimen, 44 mm. long (U.S.N.M. No. 108870; fig. 22), taken on a sloping bank in the upper of the deep holes in Bird Key reef flats. Only one other individual was seen, among the dead corals on Bird Key reef at low tide mark.

This is a fish of sandy bottom. It beds itself so expeditiously that it is impossible to see quite how this is done, as it requires a mere shiver to conceal itself. The fact that it may sink horizontally, however, implies the use of the long, strong anal, as in *Gillellus semicinctus,* to supplement the work of pectoral and ventral fins. Still it will prove, I believe, to inhabit not a fine sandy bottom, but one mixed with *Acropora* fragments or their equivalent. Both specimens observed were on such bottom, where their color combination repeated faithfully that on bits of the dead staghorn coral overgrown by small patches of incrusting corallines.

Head 3.9; depth 6.2. Eye small, prominent, 5.0 in head, twice interorbital space. D. III-XIII, 16; A. II, 25; V. I, 3, spine small, not externally visible, closely appressed to base of 1st ray, rays long, strong, exserted up to nearly half their length. Scales 3-39-9; lateral line descending abruptly to mid-level on side between 19th and 23d scales, nowhere less than 1 row of scales between it and base of dorsal fin. Teeth small, in a single series laterally. Gill rakers obsolete; pseudobranchiae none; anterior nostril tubular.

Thirteen pointed dermal processes on either lip, growing shorter laterally, form a grille before the oral cleft. Eight dermal processes on the opercular margin form a similar screen over the upper part of the gill opening, and 5 intermediate ones stand at the ends of distinct striae, raised upon the inner surface of the opercle. The remainder of the opercular margin is membranous, is expanded greatly, and covers the bases of the pectoral and ventral fins.

The ground everywhere on the back and sides is cream color, which extends beneath the tip of the jaw, upon the small first dorsal fin, and as clear white upon the basal half of the pectorals. The fins otherwise are colorless. A maroon bar across the interorbital space includes the eyes and expands below upon the cheek, where it tends to break up into three broadly diverging lines. It is faintly mottled above with gray, the general pattern extending to the iridial border with extensive overlapping of the deeper pigmented layer upon the eye proper by that on its outer envelope. Three other bars of the same rich color, somewhat browner toward the border, extend from the base of the 9th to 11th spines, the 6th to 10th rays, and from the end of the dorsal to the base of the caudal. All are narrower below, the last two only forming complete girdles. Besides these there is a narrow dusky shade, now faint now clear, extending from opercular cleft to opercular cleft by way of the base of the 4th spine.

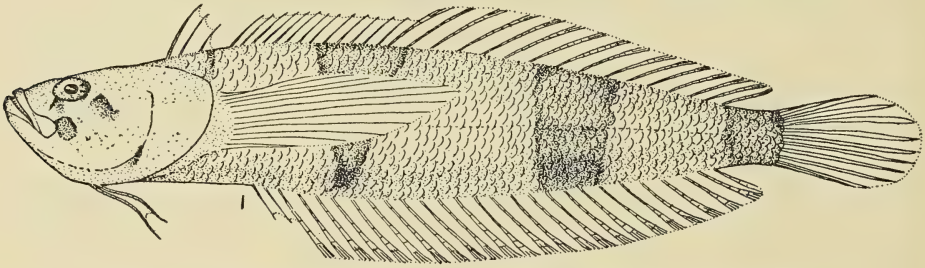

FIG. 22. *Gillellus rubrocinctus* Longley. From the type, 44 mm. long.

From *Gillellus semicinctus,* the only other species of the genus known from the West Indian region, this one is very readily distinguished by its smaller number of fin supports, its different squamation, and its less elaborate color pattern. W. H. L.

The color of the preserved specimen is pale. The four "maroon" bands mentioned by Dr. Longley remain distinct, but have become dark brown. The dusky shade across the occiput, extending from one gill opening to the other, also remains visible. The pectoral fins are white, and the other fins are colorless.

This species was recognized by Beebe and Hollister (Zoologica, vol. XIX, 1935, p. 222, fig. 27) as related to their *G. quadrocinctus* from Union Island, Grenadines, British West Indies. They stated concerning their species: "It is closest to Longley's recently described *Gillellus rubrocinctus* of unknown length, taken in Florida, but is shorter and more robust and with a smaller eye. It differs radically in color, the bands being maroon in the Florida fish, but black in our specimen. Ours has in addition four instead of three post-cephalic bands, and these differ in extent, our second body band being about three times as wide as the corresponding one in *rubrocinctus*." S. F. H.

Emblemariopsis Longley

Emblemariopsis Longley, Carnegie Inst. Wash. Year Book No. 26, 1927, p. 222 *(E. diaphana* Longley).

In *Emblemaria* the dorsal fin is enormously developed. In *Emblemariopsis* its proportions are normal, the anterior spines little elevated. Beyond this little distinguishes the two. W. H. L.

Emblemariopsis diaphana Longley

(Plate 1, figure 1)

Emblemariopsis diaphana Longley, Carnegie Inst. Wash. Year Book No. 26, 1927, p. 223—Tortugas, Florida.

The species is nowhere common. Actually it has been seen only upon *Orbicella* heads or sea fans growing on them. It is usually found fully exposed, but sometimes sheltered in the empty tubes of the worm *Spirobranchus tricornis*, the empty bored holes of the mussel *Lithodomus,* or the empty galls of a cirriped found in the coral.

Head 3.8; depth 5.9; eye 3.8. In a series of 8 specimens the fin formulae are: D. XXI, 12 or 13; A. II, 22. Body slender, compressed, tapering; snout short; mouth large, nearly horizontal, lips fleshy and reflected. Outer teeth in upper jaw slightly incurved canines, larger anteriorly than laterally; lower jaw with similar but smaller teeth; each jaw with a patch of small, pointed teeth anteriorly; teeth in a single row on palatines and vomer. Pectoral fins broad and long, reaching 2d anal ray; ventrals scarcely reaching vent; a very slight notch behind 3d dorsal spine in female; caudal short and rounded; no ocular or nuchal cirri.

In life the female is largely translucent. Only the head and trunk to the pectoral base inclusive have pigment in quantity and distribution approaching that usual in fishes. Even the head is more or less translucent brownish olive with minute white points in an external color pattern, through which in dorsal view there may be seen an internal pattern of white lines upon the pale brown of the meninges.

The dorsal membrane to the 2d or 3d spine is pigmented. Exclusive of sparsely scattered white points and faint saddles at the base of single dorsal spines or hollow rectangles with their longer sides passing through the bases of two adjacent spines, there is almost no pigment on the trunk. The saddles extend laterally scarcely more than the distance between two adjacent fin supports.

The pupillary margin of the iris is narrowly brassy. Its external boundary is very difficult to discover. The gray and brown sectors upon it extend to the sclerotic, where with the microscope the pigmentation is clearly visible through the envelope of the eye. Since this is not inflected at the orbital margin, the pattern of the head is carried directly to the border of the cornea, and the pattern at the two levels is indistinguishable with the naked eye. This condition obscures movements of the eyes, which may be rolled widely without breaking the continuity of color.

The outer surface of the peritoneum is as distinctly marked with an irregular mottling or marbling of white and brown as the skin of fishes usually is. Almost all else that the naked eye clearly perceives behind the pectoral arch is the series of brown spots along the axis of the body and upon the deep supports (radialia) of the dorsal and anal fins.

The adult males are more heavily pigmented than the females. Their black heads, protruding from the holes in which they are usually taken, are unmistakable. When these fish, however, are placed in white dishes, in a short time they become almost as light as the females. This lighter phase is that in which both sexes are usually seen in the open. Under the conditions in which they live it is highly obliterative, as the background color shimmers through the pellucid flesh. Their own color, such as it is, is the dominant color of their surroundings. Even when viewed most closely they seem mere wraiths of fishes with normal heads, each trailing a fine line of spots along its vertebral axis.

The hiding places already described serve also as brood chambers for the eggs, which are laid there. Till hatching they remain in care of the male, who posts himself in the chamber with them. Through June at least his black face is the surest indication of their presence.

A nest from one of the barnacle's galls contained about 250 eggs. These were spherical, about 0.85 mm. in diameter, and in several stages of development. Half the eggs held advanced embryos, a quarter of them contained younger embryos, and some seemed not to have begun to develop. W. H. L.

The type of the species, a male 32 mm. long, is deposited in the U. S. National Museum under catalogue number 88628.

The Tortugas collection contains 6 specimens in addition to the type, ranging in length from 23 to 28 mm. It is not known that the proportions given above by Dr. Longley are based on the type. If so, my measurements do not quite agree, perhaps owing to a slightly different method of measurement and to shrinkage since the earlier measurements were made. Dr. Longley stated that the type is 32 mm. long. According to my measurements, it is now only 30 mm. long.

The following proportions and counts are based on three specimens, respectively 30, 27, and 25 mm. long; the first one is the type, and its proportions and counts are given first. Head in standard length 4.1, 4.2, 4.1; depth 6.2, 6.8, 7.0. Eye in head 4.3, 4.6, 4.5; snout 4.8, 4.2, 4.5; maxillary 2.0, 2.2, 2.0; pectoral 1.2, 1.1, 1.1. D. XX, 13, XXI, 13, XX, 13; A. II, 22, II, 22, II, 22; P. 13, 13, 13. S. F. H.

Acanthemblemaria aspera (Longley)

Paremblemaria aspera Longley, Carnegie Inst. Wash. Year Book No. 26, 1927, p. 224—Tortugas, Florida.[3]

Acanthemblemaria arborescens Beebe and Tee-Van, Zoologica, vol. 10, 1928, p. 244, with fig.—Port-au-Prince, Haiti.

Acanthemblemaria spinosa Longley, Carnegie Inst. Wash. Year Book No. 30, 1931, p. 385 (not of Metzelaar).

At Tortugas at any hour of the day a diver may find this species "sitting" with something less than its whole head protruding from the mouth of very small holes in dead corals, bored by clams and long empty. It may be taken easily if one is provided with diving apparatus, a syringe full of copper sulphate solution, a large test tube, and a bit of soft cloth. A little of the solution is squirted into the fish's hiding place, the tube is quickly placed over the opening, the crack between the lip of the tube and the rough coral surface is closed with the cloth, and in a moment the fish leaves its burrow and swims into the tube. It attains a length of at least 34 mm. Its food consists largely of amphipods and copepods.

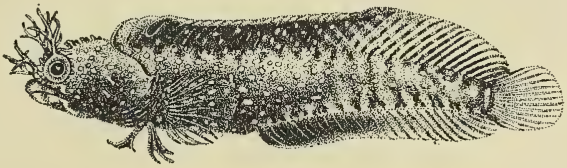

FIG. 23. *Acanthemblemaria aspera* (Longley). From a male specimen of unknown length, probably about 33 mm. (Drawing by Manson Valentine.)

In a suite of 16 Tortugas specimens the fin formulae vary as follows:

D.	XX, 16;	A.	II, 24	D.	XXI, 16;	A.	II, 24
	XX, 17;		II, 23		XXI, 16;		II, 24
	XX, 17;		II, 24		XXI, 16;		II, 24
	XX, 18;		II, 24		XXI, 16;		II, 24
	XX, 19;		II, 25		XXI, 16;		II, 25
	XXI, 15;		II, 24		XXI, 18;		II, 25
	XXI, 16;		II, 23		XXII, 15;		II, 24
	XXI, 16;		II, 24		XXII, 16;		II, 25

Body slender, compressed, tapering; profile steep; snout short; lips reflected, fleshy; maxillaries flattened at tip and extending little beyond posterior border of orbit. Outer teeth of upper jaw strong, blunt canines, supplemented in front by a patch of smaller ones within; a similar arrangement in lower jaw; 9 or 10 strong, conical teeth in a ring on vomer; double row of strong teeth on palatines. Orbital border strongly serrate anteriorly and posterodorsally; with a broad-based, blunt spine above; 2 pairs of strong spines on nasal bones; preorbital with a double and crenulate border; a pair of strong, sharp coronal spines, and lateral to these a row usually with 3 spines, diminishing posteriorly and converging toward occiput.

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[3] In their recent paper, George S. Myers and Earl D. Reid (Hancock Pacific Exped., vol. 2, no. 2, 1936, pp. 7–10) appear to have overlooked this name and description by Longley.

Dorsal fin beginning above posterior margin of preopercle, not elevated in either sex, its longest spine less than longest soft rays; pectorals much longer than ventrals but barely reaching vent; ventrals I, 3, spine very small, web of fin less excavated between rays in male than in female, making fin appear heavier in male and marking sex. A pair of multifid cirri on nasal tubes, and a pair on upper surface of eye, larger and even more freely branched (fig. 23).

Not only in the respect mentioned, but in others, the sexes of this species differ. The female shows very distinctly a set of organs which I surmise may be tactile, and of use in feeling out the surface on which she deposits her eggs. These organs consist of thin papillae, about four times as long as thick, standing in a circle about the vent, each irrigated by a visible blood vessel. The male has no comparable structure. A short, blunt cone just behind marks the opening of the excretory system. I do not see and cannot open any pore through which eggs may actually be extruded.

The differences in color also distinguish the sexes clearly. The male is on the whole much more heavily pigmented and blacker, or at least in some color phases may be much blacker, for he is very changeable. When his chromatophores are most contracted he is almost colorless except over the head and fore part of the body. The ocellus between the 3d and 4th spines marks him too. In the female only the slightest concentration of chromatophores occurs in the same place, and no trace of ocellation.

The female, like the male, is changeable in shade. Her color has usually a yellow cast, ranging from cream to straw color or pale olive. Even more than in the male, from the conspicuousness of her white chromatophores (iridiophores or guanophores), her pattern in some lights seems netted faintly about clear spaces. Her conspicuous external dark marks are not many, consisting of three broken lines under the lower jaw and throat, several spots on the operculum, and two or three on the base of the pectoral. There also are twelve or fourteen dark spots on the back at the base of the dorsal fin with inward extensions shimmering through the flesh from the median plane. Ten more lie along the base of the anal fin with similar inward extensions. Continuing the series forward are two bars on the peritoneum between pectoral axil and anus. There are also a few spots of dark pigment along the spinal cord, which is otherwise light-colored. In her palest color phases the female's visible color pattern is the deep or internal one.

The fin formulae of Beebe and Tee-Van's four specimens of *A. arborescens* are as follows. One of the two with 23 anal rays is the type.

D. XX, 15;	A. II, 22		D. XXI, 15;	A. II, 23
XXI, 14;	II, 22		XXI, 15;	II, 23

The sample is small, but it seems almost certain that even in small samples, though perhaps not as individuals, the Haitian and Tortugas fishes will be readily separable. They promise to illustrate again differentiation of local races in a population divided by the considerable barrier of the Florida Strait.

The eggs are laid in the shelters occupied by the fish. In the bottom of one 30 mm. deep were a pair of clamshells 17 mm. long, all that remained of the creature which initially prepared the site. Just external to these, and attached to the wall, were 15 eggs, each about 1.25 mm. in diameter, spherical, transparent, with a single oil globule, surrounded by a colorless and delicate membrane. The yolk was granular, and of the color of amber. All were in the same stage of development. The embryos are hardy. Some which had been left for more than 20 hours in a small dish of water with broken bits of coral and organic waste were still living. The breeding season includes at least the months of June and July. W. H. L.

The type of *Paremblemaria aspera* is a male, 33 mm. long, and bears U. S. National Museum catalogue number 88629.

The following proportions and counts are based on the type and a female paratype, 28 mm. long. The numbers given first apply to the type. Head in standard length 4.25, 4.0; depth 7.0, 7.25. Eye in head 5.1, 4.3; maxillary 2.25, 2.4; pectoral 1.75, 2.0. D. XXI, 16, XXI, 16; A. II, 24, II, 24. S. F. H.

Hemiemblemaria Longley and Hildebrand, n. gen.

Head and body compressed as in *Emblemaria;* not eel-like. Teeth in jaws anteriorly in broad bands, but in a single series laterally; in a band on vomer; and in a single series on palatines, the palatine teeth being separated from the vomerine ones by an interspace. Gill opening large, membranes broadly united and free from isthmus; the two anterior branchiostegals produced upward, extending on shoulder beyond gill opening, forming a flap with their membranes. No lateral line; no cirrus over eye or at nape. Dorsal fin not high, continuous, without definite notch, with none of the spines or rays produced; ventral with I, 3 rays. S. F. H.

Hemiemblemaria simulus Longley and Hildebrand, n. sp. MIMICKING BLENNY

(Plate 1, figures *2a, 2b*)

This species, though not numerous, is widely distributed in the Tortugas. Although a search was made for them for several years, not more than one was seen at a time. It was found almost exclusively about large coral heads, where it usually was seen with its whole head protruding from the burrow of some dead boring mollusk.

When the fish becomes attached to a particular place it may be found there day after day, even though the place has been dynamited. Unlike other local blennies, it often leaves the bottom and swims upward, where it mingles freely with such fishes as *Halichoeres bivittatus* and *Thalassoma bifasciatum*. Its motions in swimming resemble those of a labrid rather than those of a blenny.

Though propelled chiefly by its pectorals, its movements are smooth and easy, in contrast with the jerky, hesitating ones of *Blennius* and *Malacoctenus,* for example. In the aquarium it was more active by day than at night. W. H. L.

The collection contains 5 specimens, ranging in length from 32 to 100 mm. A specimen 98 mm. long (83 mm. to base of caudal) has been selected as type. It is deposited in the U. S. National Museum under catalogue number 108889.

The following proportions and counts are based on the 5 specimens at hand, the figures pertaining to the type being enclosed in parentheses: Head in standard length 3.4 to 3.8 (3.7); depth 5.7 to 6.2 (5.9); eye in head 4.4 to 6.7 (6.0); snout 4.0 to 5.7 (5.0); maxillary 2.0 to 2.15 (2.1); interorbital 7.1 to 10 (7.5); ventral 2.2 to 2.3 (2.2); pectoral 1.75 to 2.25 (1.9). D. XXIII to XXV, 15 or 16 (XXV, 15);[4] A. II, 23 or 24 (II, 23); P. 14 (14); V. I, 3 (I, 3).

Body elongate, compressed; head compressed, nearly as deep as body; eye moderate; snout rather long, pointed; lower jaws slightly projecting; mouth large, somewhat oblique; maxillary narrow, reaching well beyond posterior margin of eye in adults. Teeth as stated in generic account; lateral series in jaws and palatine teeth larger than those in anterior bands on jaws and on vomer; tongue long, narrow, and largely free; gill opening large, membranes broadly united and free from isthmus; a short dermal flap attached to anterior nostril, none over eye or at nape; dorsal spines, except 1st and 2d, which are lower, of about uniform height, and not much shorter than the soft rays; caudal broadly rounded; anal rays increasing gradually in length posteriorly, longest ones about equal in length to longest soft rays of dorsal; ventrals inserted well in advance of pectorals, under and more or less behind free margin of gill membranes; pectorals rather broadly rounded, reaching only a little more than half the distance to origin of anal.

This fish, according to Dr. Longley's field notes, is variable in color, at times appearing in a yellow phase with a broad dark band on the side, extending from the snout through the eye to the base of the caudal, and a median stripe of the same color on the back. The dark lateral band may be missing, however, behind the opercle. If so the yellow of the upper part of the side and back extends downward and shades directly into the white of the belly. Again, the dark lateral band may be broken into vertically elongate rectangles, separated by light interspaces, which may be wider than the eye.

The first-mentioned pattern is the same as that displayed by *Thalassoma bifasciatum* in the environment in which the two species commonly were seen together. The resemblance then is so great it would be extremely easy to mistake the blenny for a labrid. The deception is the more likely, because this blenny resembles *Thalassoma* in shape and form. The alternative patterns of this blenny are also alternative patterns of *Thalassoma*. Its yellower phase is very much like that phase of *Thalassoma* classified for a time as *nitidum*, and the checkered phase is like that of young *bifasciatum* when resting on the bottom.

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[4] To distinguish between spines and articulated rays in the dorsal fin of this blenny is difficult and requires a good binocular microscope and good light. Perhaps because of this difficulty, some of Dr. Longley's counts of spines and soft rays found in his notes, and based on the specimens now at hand, or others, do not quite agree with mine, though the total numbers agree. Thus, Dr. Longley's counts range from XXI, 19 to XXIV, 16.—S. F. H.

Concerning the color and habits of this blenny in relation to *Thalassoma bifasciatum,* Dr. Longley has the following: "Even more remarkable than that it mimics the color of this species is the peculiar bionomic relation with other species, for it seems to be a more or less tolerated form like *T. bifasciatum,* whose common exercise is the removal of ectoparasites from other fishes. The whole case, in short, reminds one decidedly of the relation alleged by some entomologists to exist between the batesian mimic and its protected model."

The preserved specimens, exclusive of a juvenile, have a dark lateral band, wider than the eye, and a median dark band on the back, as shown in plate 1, figure 2*a*. The yellow of the back, of course, has faded, and is pale like the belly. The dorsal is black at the base, except posteriorly in the type, though not in the other specimens, and there is a black spot between the 3d and 5th spines. This spot is present also in the paratypes, but is variable in size. The fins otherwise are colorless. The single juvenile, 32 mm. long, is paler than the other specimens. The dark lateral band becomes very narrow behind the vertical from the origin of the anal, broadening again in a very prominent black caudal spot. The dark band on the back is present anteriorly only, and the black spot of the adults on the anterior part of the dorsal already is evident.

The eggs were found on June 9, 1927, adhering to the inside of a molluscan burrow, 28 mm. in diameter, guarded by the male. The eggs were spherical and, though not measured under the microscope, were about 1.25 mm. in diameter. Each egg contained one large oil globule and many smaller ones. The eggs were rather close together, some of them even touching one another. They were in two stages of development: one batch was about ready to hatch, and in the other the head and tail were not yet free from the yolk.

Dr. Longley recognized this blenny, to which he referred throughout the many years of his investigation as the "mimicking blenny," as representing a new genus and species. Though he had proposed the specific name *simulus* in his notes, he did not mention a generic name, which was supplied by me.

The descriptions and the foregoing remarks concerning this species are based in part on the specimens in the collection, and in part on Dr. Longley's notes and a rough draft of manuscript. S. F. H.

Microdesmus floridanus (Longley)

Cerdale floridana Longley, Carnegie Inst. Wash. Year Book No. 33, 1934, p. 258—Tortugas, Florida.

Microdesmus floridanus Reid, Proc. U. S. Nat. Mus., vol. 84, 1936, p. 60, figs. 9*a*, 10*b*, and pl. 2, fig. 2.

Known from several specimens, all from Tortugas. One was collected on Bird Key reef, one swimming about a light in the channel between Garden Key and White Shoal, another was incidentally driven out with formaldehyde from among loose fragments of coral about the burrow of *Opisthognathus aurifrons* in 2 fathoms of water on the northern end of Loggerhead bank.

Length of type 66 mm., catalogued in the U. S. National Museum under number 102050 (fig. 24). From examination of this and other specimens it appears that the head is contained 6.2 to 6.7 times in the length to the base of the caudal fin; depth 7.5 to 7.8; eye 5.0 in head. D. 44 to 46, anterior 13 or 14 fin supports simple, weak, tips filamentous, the remainder branched, articulate; A. 30, in female at least all branched, its origin under 3d dorsal ray; dorsal and anal fins united with base of caudal; V. I, 3, spine weak, filiform; P. 13; C. 17, rounded, with additional reduced rays at dorsal and ventral margins. Scales very small. Teeth in lower jaw short, blunt canines, close-set, regular, of approximately equal size, in 2 rows. Gill openings narrow, below and before base of pectorals.

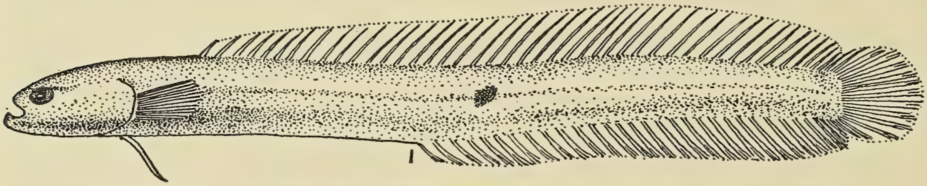

Fig. 24. *Microdesmus floridanus* (Longley). From the type, 66 mm. long.

Vent decidedly more remote from snout than from base of caudal. Genital papilla in female conspicuous, fleshy, with so large an opening as to suggest the possibility of viviparity.

In coloration pale, scarcely darker than straw color. Body finely and uniformly speckled above with stellate chromatophores, lower parts with none, fins immaculate. W. H. L.

Reid (see citation above) included some additional information in his description, wherein he stated that the body and head, except the snout and the lower jaw, are covered with minute nonimbricate scales embedded in the skin, each scale having 30 radiating striae. Myomeres were given as 20 on the body and 23 on the caudal. Vertebrae (determined by X-ray photographs) 20 body and 24 caudal.

I have examined the type (U.S.N.M. No. 102050), which now has a total length of 63 mm. and standard length of 56 mm. The difference between this length and that given by Dr. Longley may be due in part at least to shrinkage in preservative. The collection contains 6 other specimens, ranging in length from 43 to 71 mm. One of these, according to the label, was taken in 10 fathoms east of White Shoal. S. F. H.

Triacanthodes lineatus Longley

Triacanthodes lineatus Longley, Carnegie Inst. Wash. Year Book No. 34, 1935, p. 88—Tortugas, Florida, in 60 to 125 fathoms.

Triacanthodes zebra Nichols and Firth, Amer. Mus. Novitates, No. 883, 1936, p. 2, fig. 1—off Cape Henry, Virginia, in 50 to 60 fathoms.

This species occurs at depths of 60 to 125 fathoms south of Tortugas, where 20 or more specimens were taken. It is sexually mature at 100 mm. or less, and it attains a length of at least 120 mm.

The following description is based on the type: Dorsal spines graduated downward, 1st almost as long as head, last equaling horizontal diameter of pupil. Ventral spines strong, extending to tip of pelvic bone, 2d ray minute, adnate to body wall. Teeth in each jaw strong, conical, in a single series except anteriorly, where there is a single pair in each jaw within the main row. Opercular cleft not extending ventrally to lower margin of pectoral base. Body covered with small scales, each with a single or several spinules, these extending upon the fluted dorsal and ventral spines to their tips.

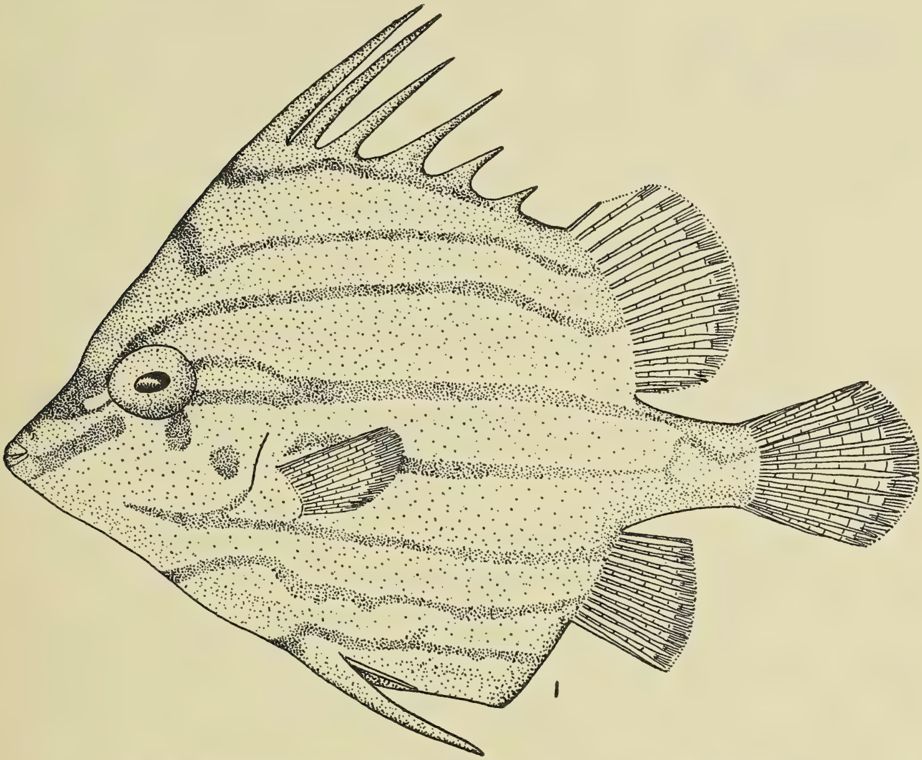

FIG. 25. *Triacanthodes lineatus* Longley. From the type, 73 mm. long.

Rather rosy dorsally in life, vertical fins faintly orange, body marked with about eight longitudineal, linear, nonanastomosing streaks of green.

The total length of the type is 73 mm.; length to base of caudal fin 57 mm.; depth 40 mm. (1.43); head 20 mm. (2.85); eye 7.0 mm. (2.85); snout 9 mm. (2.25); first dorsal spine 20 mm. (1.0); ventral spine 17 mm. (1.18); pectoral 10 mm. (2.0); caudal peduncle 6 mm. (3.33); snout to origin of dorsal 30 mm. (1.85). D. VI, 16; A. 14; P. 13; V. I, 2. It is deposited in the U. S. National Museum under catalogue number 101319 (fig. 25). W. H. L.

Of the 20 or more specimens taken, Dr. Longley's collection now contains, in addition to the type, 10 specimens, ranging in length from 65 to 122 mm. The smaller specimens are shorter and deeper than the larger ones, and apparently were regarded as a distinct species for some time by Dr. Longley, which seems to be the reason for choosing a small (young) specimen as type. The young, at least in the preserved material, also are more distinctly marked with longitudinal stripes.

It seems desirable to state the length and proportionate depth for each specimen measured, to show the apparently gradual decrease in proportionate depth as the fish grows. The lengths are stated in millimeters: 65, 1.5; 73, 1.5; 77, 1.55; 97, 1.65; 104, 1.7; 110, 1.8; 113, 1.9; 116, 2.0; and 122, 2.0. The difference in depth with age is reflected also in the comparatively great difference in depth of caudal peduncle. The range in proportions and counts of 10 specimens follow: Head in standard length 2.65 to 2.85; depth 1.5 to 2.0; snout to origin of dorsal 1.9 to 2.05; snout to base of ventral 2.05 to 2.25. Eye in head 2.5 to 2.8; snout 2.0 to 2.25; caudal peduncle 3.2 to 4.0; first dorsal spine 1.0 to 1.15; ventral spine 1.25 to 1.4; pectoral 2.0 to 2.5. D. VI, 15 or 16; A. 13 or 14; P. 13 (once 12).

The color in alcohol varies from brown to straw color, the smaller specimens being darker than the larger ones. The longitudinal stripes, which were green in life, remain more or less visible in all the preserved specimens, but are most distinct in the smaller ones, and are dusky in color. The fin spines correspond in color to the portion of the body to which they are attached, and the soft fins are plain translucent.

Although I have not seen the type of *T. zebra* Nichols and Firth (see citation above), I am rather confident from an examination of the description and figure that it is identical with *T. lineatus,* and accordingly I have placed the name in synonymy.

Breder (Bingham Oceanog. Coll., vol. 1, 1927, p. 75, fig. 33) has figured but not described a specimen from the Caribbean Sea, which he identified as *Hollardia hollardi.* It is plainly not that genus or species, if the figure is correct. Among other characters it differs prominently, as is evident from comparing the figure with the type, a skin in fairly good condition in the U. S. National Museum (No. 15454), in the much more anteriorly placed dorsal fin, which has its origin, according to the figure, over or slightly in advance of the base of the pectorals. In the type of *H. hollardi,* the origin of the dorsal is behind the base of the pectoral, and even somewhat behind the base of the ventral. I cannot identify Breder's specimen (or specimens) with *T. lineatus,* because the color pattern disagrees, and according to the figure the soft dorsal seems to have too few rays. As already pointed out by Myers (Smithsonian Misc. Coll., vol. 91, 1934, p. 8), it probably represents a distinct species. S. F. H.

Alutera ventralis Longley

Alutera ventralis Longley, Carnegie Inst. Wash. Year Book No. 34, 1935, p. 89—Tortugas, Florida.

The young of this species and of *A. schoepfii* were taken together in floating *Sargassum* in overlapping sizes and very like one another in behavior. Both undergo changes in proportion as they grow, and an appreciation of their differences for this additional reason develops slowly. But when check series through the critical sizes have once been segregated and the norms for the two species established, they are easily and accurately separable.

The retrorse barbs on the first dorsal spine of the present species are fewer and stronger, the spine itself is shorter, stouter, regularly shaped to the tip; not thin and spindling with scarcely the same shape in two individuals, as in *A. schoepfii.*

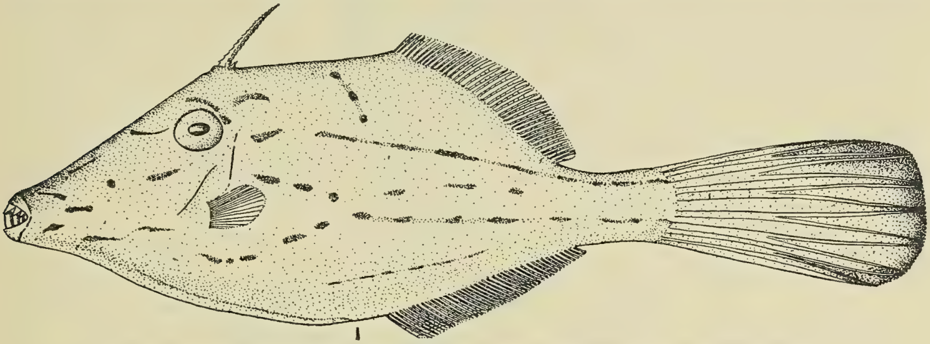

FIG. 26. *Alutera ventralis* Longley. From the lectotype, 120 mm. long.

The fish has on the average about 4 more rays in the soft dorsal, 3 more in the anal than *A. schoepfii.* At the same standard length it is considerably the deeper of the two.

Seated on the long, falcate pelvic bone, where it turns abruptly toward the anus—shaping a ventral contour less even and gently sloping than that in the young *A. schoepfii*—is an obscure but unmistakable vestige of the reduced ventral spine of *Monacanthus.* Microscopic in size, it is scarcely larger than a sixth of the fish's minute scales and is freely movable in the skin.

A specimen 140 mm. long, standard length 100 mm., dead and neglected for some time, clearly had a pattern much like that of *Alutera scripta* in its checkerboard design and blue penciling.

DEPTH IN STANDARD LENGTH, TOGETHER WITH THE NUMBER OF DORSAL AND ANAL RAYS IN TEN SPECIMENS OF ALUTERA VENTRALIS

Standard length (mm.)	Depth (mm.)	Depth in standard length	Dorsal rays	Anal rays
32	9.5	3.4	39	40
40	12	3.3	40	42
44	14	3.1	38	39
45	15	3.0	37	42
55	25	2.2	40	39
59	22	2.7	39	41
63	27	2.3	40	42
68	26	2.6	39	42
83	34	2.4	39	42
100	40	2.5	40	41

Three specimens not in the table had in the dorsal, 39, 41, and 40 soft rays, and in the anal 40, 40, and 42 rays.

W. H. L.

The collection contains 17 specimens, ranging in length from 45 to 130 mm. Although Dr. Longley stated in the original account (see reference above) that the type, a specimen 120 mm. long, 78 mm. to the base of the caudal, was deposited in the U. S. National Museum, no record was found there. The collection contains a specimen, unlabeled, found with four others, taken in "Channel west of White Shoal, June 22, 1932" that is of the size given. It cannot be stated definitely, however, that this was the specimen selected as type by Dr. Longley. Therefore, upon my selection this specimen is designated lectotype, and it bears U. S. National Museum catalogue number 109177 (fig. 26).

The following proportions are based on 3 specimens, respectively 80, 85, and 90 mm. long, 52, 55, and 63 mm. to the base of the caudal, and on the lectotype. The proportions enclosed in parentheses apply to the lectotype. Head, measured to upper angle of gill slit, in standard length 2.9, 2.9, 3.0 (2.9); depth, measured at dorsal spine, 2.2, 2.25, 2.3 (2.2); snout to base of dorsal spine 2.7, 3.0, 2.7 (2.9). Eye in head 4.0, 4.3, 4.2 (4.5); snout 1.2, 1.3, 1.1 (1.2); interorbital 4.75, 6.0, 4.4 (4.6); caudal peduncle 3.1, 3.3, 3.0 (3.4). The lectotype has 39 soft rays in the dorsal and 42 in the anal, these counts being within the range given in the accompanying table. The specimens measured by Dr. Longley (see table) appear more slender than the ones measured by me only because he measured the depth at the origins of the second dorsal and anal, where the body is not so deep as at the dorsal spine, where I measured.

The color of preserved specimens is very variable, ranging from uniform pale brown or grayish to plain dark brown. Some specimens have dark spots along the back only, whereas others are more profusely spotted or blotched, the blotches sometimes forming more or less distinct bands. The lectotype is plain light brown. This species apparently does not differ in color from *A. schoepfii.*

S. F. H.

Sphoeroides dorsalis Longley

Sphoeroides dorsalis Longley, Carnegie Inst. Wash. Year Book No. 33, 1934, p. 259—Tortugas, Florida.

Tetrodon (Spheroides) harperi Metzelaar, Trop. Atl. Vissch., 1919, p. 170—St. Eustatius (not of Nichols).

Spheroides marmoratus Breder, Bull. Bingham Oceanog. Coll., vol. 1, 1927, p. 79—Green Cay, Bahamas (not of Ranzani).

This fish was taken occasionally in 10-fathom channels within the lagoon, rarely in 40 fathoms south of Tortugas, and east of Bush Key and Bird Key reef in 13 to 14 fathoms, where it is not uncommon. The largest seen did not exceed 140 mm. in length.

Pectoral rays commonly 16 or 17, upper ray short, in length equal to about one-quarter vertical height of fin base; D. 8; A. 7; 1st ray in each fin longer than base of fin; anal origin beneath middle of dorsal; the expanding caudal truncate with prominent angles, the upper slightly exserted, with posterior margin of folded fin distinctly excavate. Upper jaw with a distinct vertical ridge on either side the median suture. In a representative specimen, 112 mm. long,

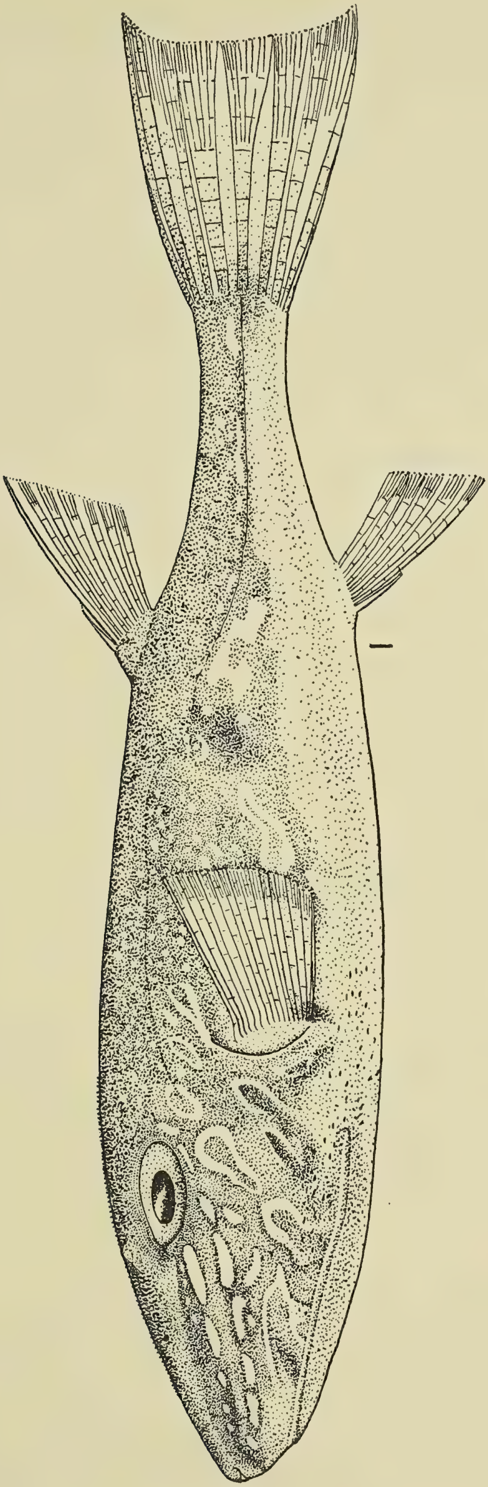

Fig. 27. *Sphoeroides dorsalis* Longley. From the lectotype, 112 mm. long.

the length to the base of the caudal is 96 mm.; depth 22 mm.; greatest breadth 19 mm.; head 33 mm.; horizontal and vertical diameters of eye 11.0 and 7.0 mm. respectively; interorbital width 3.0 mm.; snout 17 mm.

On the dorsal surface, in the vertical plane in which the main trunk of the lateral line rises highest, there are two small fleshy lappets about 1.0 mm. long and 5.0 mm. apart, and no others. From a point between them to the tip of the premaxillary processes the dorsal surface is covered thickly, for the most part, with prickles up to 1.0 mm. in length. They stand in a band which includes the nostrils and the envelope of the eye between the orbital processes. Between the posterior orbital margins they are strongest and 5- or 6-rooted. On the belly are others extending as far forward, and posteriorly reaching but not passing the vertical of the pectoral tip. The remainder of the body is naked.

The dorsal surface is olivaceous, much mottled with brown; belly white. The lower part of the side shows anteriorly much pale yellow, with pale blue vertical pencilings upon the cheek and before the gill openings. The pattern is subject to some change. In one phase a broad, dark streak extends from the angle of the mouth to the lower margin of the orbit. At the same time a vague dark area may show before the opercular cleft; also two dusky saddles, of which the second includes the base of the dorsal fin. Low down on the side, a little in advance of the dorsal fin, there is a permanent dark nucleus of the size of the eye, which is somewhat elongated vertically. The caudal fin is dusky, with half its surface covered by a pale vertical band rather nearer its base than its extremity.

A specimen having a total length of 25 mm. (Mus. Comp. Zoöl. No. 26173), taken by the *Blake* at 22° 44′ N., 83° 26′ W., though it has only four dorsal rays, seems clearly enough to belong to this species. Its pectoral rays are 16, its anal 7; and the two dorsal lappets are distinctly visible.

Three small specimens collected by Boeke at St. Eustatius, and referred by Metzelaar to *S. harperi* (see reference above), have D. 8; A. 7; P. 17. The first ray in the dorsal and anal fins equals or exceeds the fin base in length. All three have the dorsal lappets of *S. dorsalis,* and no others.

Each of 30 specimens, 24 to 32 mm. long, selected at random from the lot of 69 collected by the First Bingham Oceanographic Expedition at Green Cay, and referred by Breder to *S. marmoratus* (see reference above), has also the two dorsal lappets of *S. dorsalis.* Of 10 more, closely examined, 8 have D. 8; A. 7; P. 17 on either side, and the remaining 2 have a ray less. In 10 examples examined, the upper pectoral ray constantly is more than half as long as the next one.

W. H. L.

The Tortugas collection contains 10 specimens, ranging in length from 93 to 130 mm., which evidently belong to this species. Two of these specimens were taken in otter-trawl hauls, one of which was made east of Bird Key reef, and the other to the southeast of Bird Key. The other specimens are without labels.

Although Dr. Longley stated in the original account of this species (see reference above) that the type was deposited in the U. S. National Museum, there seems to be no record of its receipt there. Neither was a specimen found in his collection that was designated as type. Therefore, it becomes my duty to select a type. Inasmuch as Dr. Longley stated that his type was 112 mm. long (99 mm. to base of caudal, which apparently is an error, as such a proportion does not exist among the specimens at hand), I have selected a specimen of that length (112 mm.), which measures 88 mm. to the base of the caudal, as the lectotype. It is deposited in the U. S. National Museum under catalogue number 109179 (fig. 27).

The following proportions and counts are based on the lectotype and two other specimens 105 and 112 mm. long (82 and 90 mm. to base of caudal). The figures pertaining to the lectotype are enclosed in parentheses. Head in standard length 2.8, 2.6 (2.7); distance from snout to origin of dorsal 1.4, 1.4 (1.4). Eye in head 4.0, 4.25 (5.2); snout 1.8, 2.0 (1.8); interorbital (bone) 9.6, 7.5 (10); caudal peduncle 5.75, 6.2 (5.75); pectoral 2.2, 2.6 (2.2). D. 8, 8 (8); A. 7, 7 (7); P. 16, 15 (16), exclusive of uppermost rudimentary ray.

The color remains essentially as described by Dr. Longley, probably the most outstanding mark being the vertically elongated dark blotch on the side.

This species differs prominently from *S. spinglerí,* the only other species of the genus taken at Tortugas, not only in color, but also in the much more prickly body, the more numerous pectoral rays, and the concave caudal fin.

S. F. H.

Ogcocephalus parvus Longley and Hildebrand, n. sp.

The collection contains 13 specimens ranging in length from 53 to 78 mm., taken at depths of 40 to 60 fathoms, south of Tortugas, where representatives were caught occasionally. The specimens seem to represent an undescribed species, for which Dr. Longley proposed the name *O. parvus.* However, he did not draw up a description. According to his notes, from which the information concerning its capture and occurrence is taken, it is sexually mature at a length of 60 mm. or less, indicating that this fish does not grow large. A specimen 78 mm. long, bearing U. S. National Museum catalogue number 109313, has been selected as type (fig. 28).

The following proportions and counts are based on 6 specimens, including the type. The numbers pertaining to the type are enclosed in parentheses. Head, measured from tip of upper jaw to gill opening, in standard length, the latter measured from tip of upper jaw to base of caudal, 1.75 to 1.9 (1.8); depth 3.3 to 3.8 (3.6); snout to vent 1.5 to 1.7 (1.6). Eye in head, the latter measured without rostral process, 5.2 to 6.75 (6.2); rostral process in advance of eye 5.2 to 6.5 (5.2); interorbital 7.3 to 9.5 (7.3); caudal peduncle 6.2 to 7.2 (6.2). D. 4 (4); A. 4 (4); P. 10 or 11 (11); C. 8 or 9 (9).

The width of the body varies greatly, according as the specimen was preserved in an expanded or contracted condition, making a width measurement virtually useless. The width and depth of the caudal peduncle are about equal. The rostral process is rather short and its extension in advance of the mouth is only a little less than its extension in advance of the eye, the anterior margin of the eye being behind the tip of the upper jaw a distance scarcely equal to the diameter of the pupil.

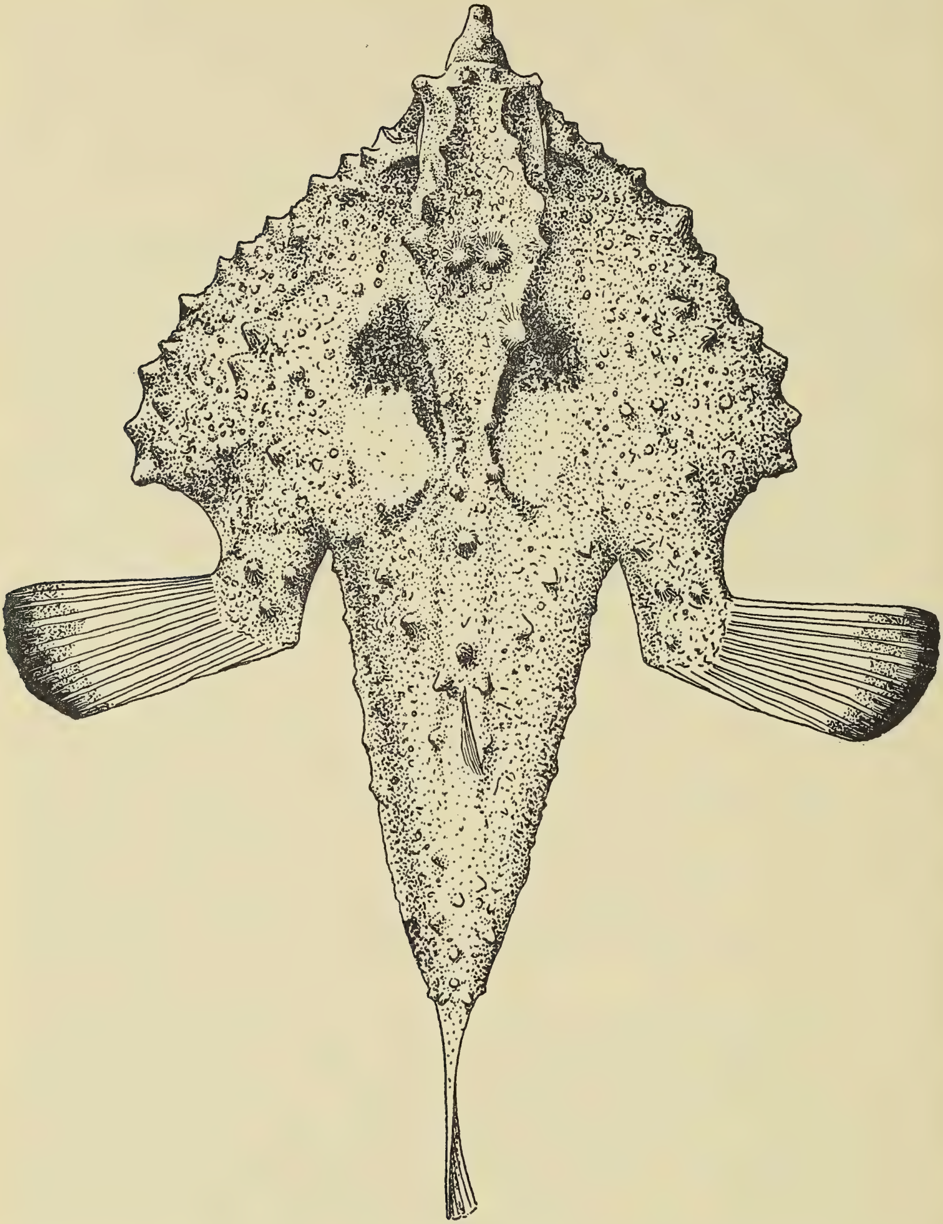

FIG. 28. *Ogcocephalus parvus* Longley and Hildebrand, n. sp. From the type, 78 mm. long.

The upper surface of the entire fish is very rough, being covered with spines and tubercles of various sizes, many of which are short and very stout, buckler-like. The largest ones occur on top of the head and on the back in advance of the dorsal, whence a series extends forward and downward to the base of the "wrist" of the pectoral, recurring and continuing on the wrist. Several irregular series of large ones are present on the anterolateral margin of the disk. Large ones are present also on the dorsal and lateral surface of the caudal peduncle. There is a rather large buckler on the anterior rim of the orbit, and three more of unequal size on its upper rim. There is a pair of rather small bucklers at the anterior end of the interorbital space and a pair of larger ones posterior to it. The ventral surface is fairly uniformly granular, though there are a few (three in the type) enlarged tubercles on the median line in advance of the anal.

Rostral tentacle three-lobed, lying within rostral cavity, which is fully as broad as long. Dorsal and anal fins small, origin of former about twice as far from tip of rostral process as from base of caudal, latter enveloped in rather heavy skin, with origin under tips of dorsal rays. Caudal broadly rounded; ventrals rather long and narrow, only a little shorter than pectorals, and inserted about twice as far from base of caudal as from tip of lower jaw; pectorals with a prominent free "wrist," rather broad, with a distally rounded margin.

The color is described in Dr. Longley's field notes as follows: "Dorsal surface irregularly blotched with red on yellow in life. Tips of pectorals black; tip of caudal red; anal also dark-tipped; ventrals pale. White patches at angle of mouth, extending on under surface, sometimes followed by others nearly as large."

The preserved specimens vary greatly in shade, some being much darker than others. The markings on the ground color are much more distinct on the lighter-colored individuals. In some of the darker specimens no markings, in fact, are discernible. In the type, which is a light-colored specimen, there are indefinite patches of brown, and the small spots on the disk consist of brown rings around the bases of the smaller spines. The lower surface is plain dirty gray. The fins remain about as described by Dr. Longley in fresh material, being pale; the tips of the pectorals and anal dark, the dark tips of the pectorals, however, being bordered below with pink, and the tips of the caudal still showing a slight tinge of red. The red is not retained, however, in all the specimens at hand.

Aside from the apparently small size attained, this species differs from the other local species in the very rough body, the spines and bucklers being numerous and many of them short and thick. It has a much shorter rostral process than the other local species, exclusive of *O. nasutus.* From the latter it differs principally in the coarser and more numerous spines on the skin, in having 11 instead of 13 pectoral rays, and in having pale spots surrounded by brown rings (in preserved specimens), instead of having dark spots on parts of the upper surface, as in *O. nasutus.* S. F. H.

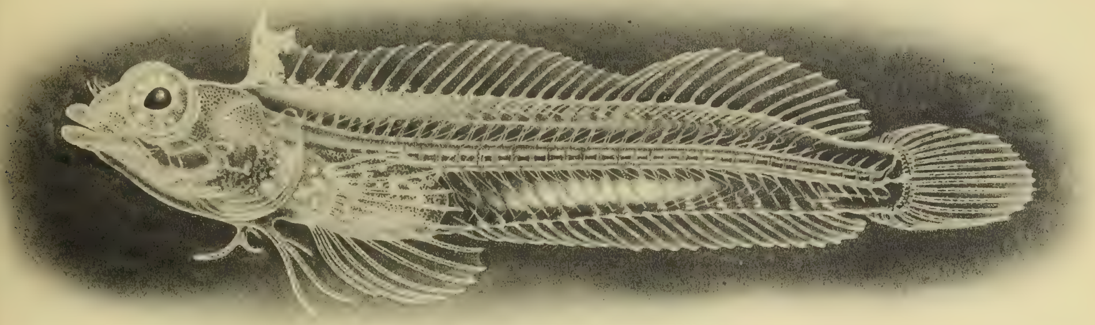

FIG. 1. *Emblemariopsis diaphana* Longley. From a specimen of unknown size, probably about 30 mm., showing internal markings in part. (From original in color, painted from life by Manson Valentine.)

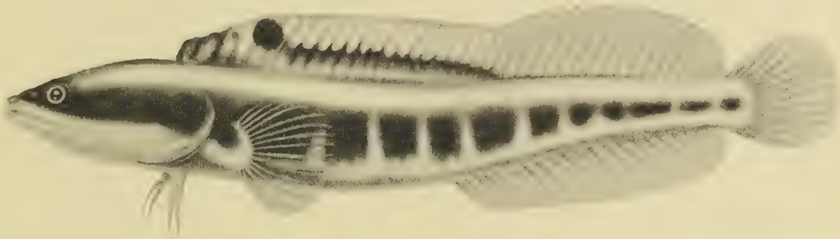

FIG. 2*a*. *Hemiemblemaria simulus* Longley & Hildebrand, n. gen. and n. sp. From a specimen of unknown size, probably about 100 mm., showing the banded phase. (From original in color, painted from life by Manson Valentine.)

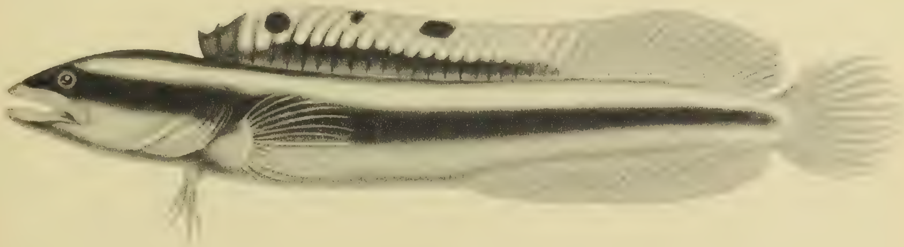

FIG. 2*b*. *Hemiemblemaria simulus* Longley & Hildebrand, n. gen. and n. sp. From a specimen of unknown size, probably about 100 mm., showing the striped phase. (From original in color, painted from life by Manson Valentine.)

PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

XV

Structure and Function of the Gut and Symbiosis with Zooxanthellae in Tridachia crispata (Oerst.) Bgh.

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With nine text figures

[ISSUED SEPTEMBER 14, 1940]

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Structure and Function of the Gut and Symbiosis with Zooxanthellae in Tridachia crispata (Oerst.) Bgh.

INTRODUCTION

During the visit of the senior author to the Tortugas Laboratory in the season of 1934, a number of specimens of a very interesting and beautiful species of opisthobranch mollusk were collected at low tide on the surface of the reefs. Superficial examination in the laboratory revealed the invariable presence of brown unicellular algae, or zooxanthellae, within their tissues. Material was fixed in a variety of ways and a number of animals were preserved intact.

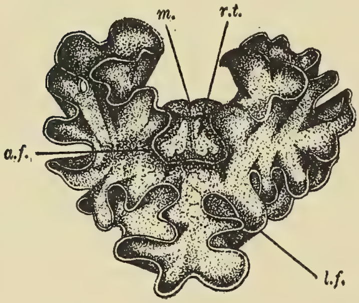
Considerable difficulty was encountered in tracing the name of the species, but eventually, by the kind offices of Professor C. H. O'Donoghue, it was identified as *Tridachia crispata* (Oerst.) Bgh., of the family Elysiidae, order Ascoglossa. These animals were placed by Pelseneer (1894, 1906) among the Nudibranchia and actually at the summit of evolution within this group, and this view was later adopted by Eliot (1910). But the majority of other workers, impressed by the primitive nature of the nervous system, considered that they should be placed at the base of the Nudibranchia and closely allied to the lophocerid Tectibranchia, which Pelseneer had included in the Bullomorpha. Thiele (1926) actually included the animals comprised in the Elysiomorpha of Pelseneer with the lophocerids in his order Sacoglossa. This relationship was clearly demonstrated by Russell (1929), who made a detailed study of the nervous systems in elysioid and aeolidioid mollusca and gives a full account of previous work and opinions. In a comparison between these systems and that of *Lobiger* (a lophocerid), she shows that, out of thirteen points of comparison, *Elysia* resembles *Lobiger* in ten but *Aeolidia* only in one, namely, the absence of an osphradium and of a ctenidium, organs which, as shown elsewhere (Hulbert and Yonge, 1937), are always in intimate functional association.

The structure of *Tridachia crispata* has been described only by Bergh (1876), who saw crudely preserved material. He states that the genus occurs only in tropical waters, two species having been collected in the Hawaiian Islands, one of dubious standing at Guadeloupe in the West Indies, and *T. crispata* in the Antilles (St. Croix) and on the coast of Venezuela. The occurrence of the latter species at the Tortugas does not appear to have been recorded previously to this paper.

Apart from the question of association with zooxanthellae, not recorded by Bergh, the point of greatest interest in this animal lies in the structure and mode of functioning of the highly specialized alimentary system, particularly in the light of recent work on these lines in *Cymbulia* (Howells, 1936), and a variety of other Tectibranchia (Fretter, 1939), in the nudibranch *Jorunna* (Millott, 1937), and in the aeolids (Graham, 1938). Investigation of this subject was accordingly undertaken, the mechanism of feeding being studied in the allied British species *Elysia viridis*. But shortly after this had been completed it was found that similar investigations had just been carried out by Dr. V. Fretter on the latter species together with two other members of the Ascoglossa, *Limapontia capitata* and *Hermaea dendritica,* and essentially identical conclusions reached. This part of the work has, therefore, been omitted, full reference being made to the results of Fretter (1940). The work of Bergh (1876) on *T. crispata* has been considerably extended, especially on the functional side, and, in places, corrected.

External Features and Habits

Tridachia crispata has the typical elysioid form but with the body so excessively flattened dorsoventrally that it is leaflike. The lateral extensions of the body are thrown into a series of undulating folds (fig. 1) which, unlike the corresponding structures in *Elysia,* are continuous anteriorly behind the head (fig. 1, *a.f.*). It is a relatively large species, attaining a length of up to 4 cm. and a width of some 2.5 cm.; the form of the body continually alters during movement. The head carries a pair of folded, flattened tentacles each of which has a small eye near the base on the outer side. In the middle line, immediately behind the anterior body fold, lies a rounded, blisterlike swelling indicating the position of the renopericardial complex (fig. 2). In life the heart can be seen beating through the thin body wall. The renal orifice (fig. 2, *r.o.*) consists of a minute opening on the right side of this. The appearance of the heart and kidney after partial dissection is shown in figure 2. The opening of the anus (*a.*), together with those



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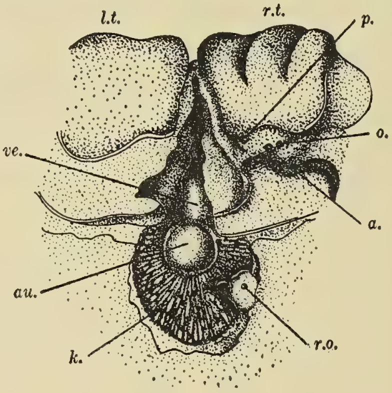

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Fig. 1. *Tridachia crispata,* dorsal view of a specimen fixed but well expanded; the head region is curled back. × 1½. *a.f.,* anterior body fold; *l.f.,* lateral body fold; *m.,* mouth; *r.t.,* right tentacle.

Fig. 2. *Tridachia crispata,* anterior end of the body viewed from the dorsal aspect, with the contents of the pericardium displayed. × 9. *a.,* anus; *au.,* auricle; *k.,* kidney; *l.t.,* left tentacle; *o.,* opening of oviduct; *p.,* opening of penis; *r.o.,* renal opening; *ve.,* ventricle. Other lettering as in figure 1.

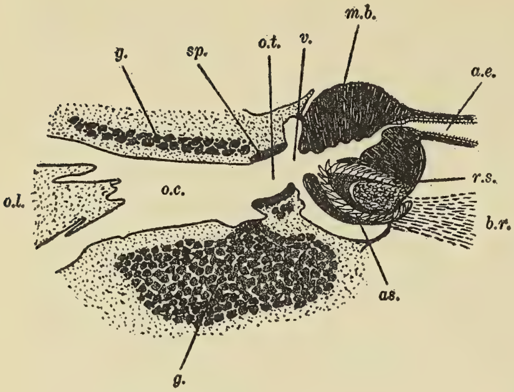
of the penis (*p.*) and oviduct (*o.*), lies immediately behind the base of the right tentacle anterior to the body fold. The third opening of the triaulic reproductive system, that of the vagina, was not found in any of the specimens, and in none of those dissected was this organ present. Presumably, therefore, these animals, although as large as any obtained, were not sexually mature. Pelseneer (1894) found that this portion of the reproductive system was absent in immature specimens of *Elysia viridis.* These animals are probably all annuals, and the vagina may not be developed until the onset of the breeding season.

The foot is flat and relatively narrow; it is clearly adapted for crawling over a hard surface. The animals are of a beautiful green color, the pigmented areas forming a delicate reticulation over the back and sides, and the ventral surface being colorless. In life a thin brown band can be seen a little distance in from the edge of the folded margins of the body. This indicates the position of the zone of zooxanthellae, which are responsible for the color. The surface of the body is slimy, the result of secretion by the great numbers of large mucous glands (fig. 8, *m.g.*), previously described by Bergh (1876).

Although probably very abundant on the surface of the reefs at the Tortugas, *T. crispata* has to be sought with care because it invariably occurs under stones. This habit may be due to the need for protection, since the animal appears devoid of any means of defense. At the same time it feeds on green algae, so that it can never venture far from some source of light. It is possible that the irregular outline of the animal together with its green color affords some measure of protection, and that, as suggested by Eliot (1910), the Elysiidae may secrete a distasteful mucus.

Structure of the Gut

The mouth (fig. 1, *m.*) is situated on the anteroventral margin of the head. In life the animal was observed to move about in a glass dish with the mouth opening widely distended and sweeping the bottom, presumably in search of food. The oral cavity (fig. 3, *o.c.*) consists of an irregular cone-shaped cavity bounded by a series of lobes (*o.l.*) and extending posterodorsally. At its apex lies the opening of the short oral tube (fig. 3, *o.t.*). In cross section the anterior region of this tube has the form of an inverted T; the horizontal limb disappears a little distance posterior to the opening, and the vertical limb dilates dorsally and communicates with the wider vestibule (figs. 3 and 4, *v.*). All these regions are ciliated. There is great development of mucous glands in the connective tissues around them. The largest masses (fig. 3, *g.*) are arranged laterally on either side of the oral cavity and open on either side of the opening of the oral tube at the extremities of the horizontal limb. There are other glands around the oral tube itself, also mucous glands in the ciliated epithelium in all regions; the pedal gland opens into a post-oral depression. Fretter (1940) considers that some of the glands around the mouth opening in *Elysia viridis* secrete a cementing substance; it is uncertain whether similar glands occur in *Tridachia.* The oral tube is surrounded by a powerful sphincter (fig. 3, *sp.*).



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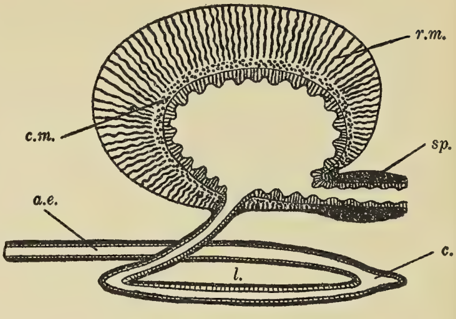

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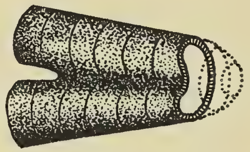

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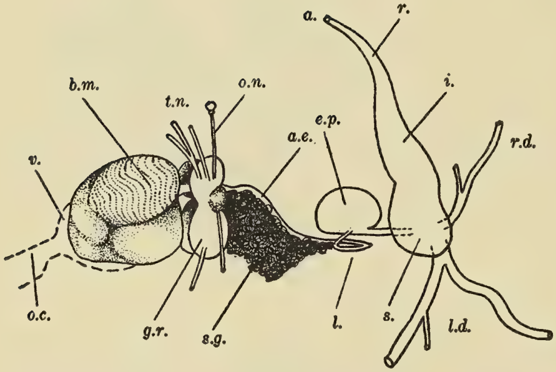

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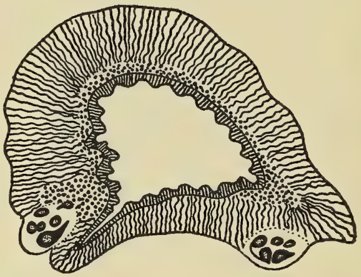

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Fig. 3. *Tridachia crispata,* longitudinal section through the anterior end of the alimentary canal, reconstructed from several sections. × 27. *a.e.,* anterior esophagus; *as.,* ascus; *b.r.,* buccal retractor (shown by broken lines indicating that it is not present in the middle line); *g.,* gland cells; *m.b.,* radiating muscles in buccal mass; *o.c.,* oral cavity; *o.l.,* oral lobe; *o.t.,* oral tube; *r.s.,* radula sac; *sp.,* sphincter muscle; *v.,* vestibule.

Fig. 4. *Tridachia crispata,* semidiagrammatic representation of the alimentary system, with the circum-esophageal ganglionic ring. × 25. *b.m.,* buccal mass; *g.r.,* ganglionic ring; *i.,* intestine; *l.,* esophageal loop; *l.d.,* left ducts of digestive diverticula; *o.n.,* optic nerve (with eye); *e.p.,* esophageal pouch; *r.,* rectum; *r.d.,* right ducts of digestive diverticula; *s.,* stomach; *s.g.,* salivary glands; *t.n.,* tentacular nerves. Other lettering as before.

Fig. 5. *Tridachia crispata,* semidiagrammatic reconstruction of the esophageal pouch, with loop and caecum viewed in longitudinal section. × 70. *c.,* caecum; *c.m.,* circular muscle; *r.m.,* radiating muscle. Other lettering as before.

Fig. 6. *Tridachia crispata,* diagram to illustrate the structure of the caecum at the anterior end of the esophageal loop.

Fig. 7. *Tridachia crispata,* transverse section through the esophageal pouch, showing the gastro-esophageal ganglia. × 70.

The wide vestibule provides communication between the oral tube and the lumen of the buccal mass (fig. 4, *b.m.*). This is a relatively large, globular organ, but its lumen is very restricted, the greater part of the bulk being due to the thickness of the radiating muscles (fig. 3, *m.b.*) which surround the thin, cuticularized epithelium. As shown in figure 3, almost half of the buccal mass anteriorly lies free within the cavity of the vestibule. A pair of stout retractor muscles (fig. 3, *b.r.*) are attached to the posteroventral margins of the buccal mass on either side of the ganglionic nerve ring (see fig. 4). Within the buccal mass lie the typical ascoglossan odontophore and radula. The latter, which has been well figured by Bergh (1876), bears a single row of strong, elongate teeth. Each is sharply pointed and also serrated on the posterior margin. The radula sac (fig. 3, *r.s.*) is situated in the middle of the buccal mass, the young teeth being directed posteriorly. At the tip of the odontophore the radula bends around ventrally and is then passed into the ascus (*as.*), which extends posteriorly below the radula sac. In this region the used, but still intact, teeth are stored. Fretter (1940) considers that the ascus represents the modified subradular sac.

The ducts of a pair of salivary glands open, one on each side, into the posterodorsal corner of the buccal cavity in the region where the esophagus passes back. The glands themselves form a lobular mass (fig. 4, *s.g.*), the two halves of which are indistinguishable, situated on either side of the esophagus posterior to the ganglionic nerve ring (fig. 4, *g.r.*). The ducts are extremely narrow but, after passing through the nerve ring and the muscles of the buccal mass, they expand somewhat into two small vesicles which are surrounded by muscle. Fretter (1940) states that in *E. viridis* the ducts are ciliated posterior to their passage through the buccal musculature but not after this. This appears to be true of *T. crispata* also. The assumption is that secretion is passed along the ducts by ciliary action posteriorly but that the vesicles act as a pump as well as a reservoir and force the secretion into the buccal cavity.

The esophagus is circular in cross section and runs posteriorly from the buccal mass through the ganglionic nerve ring as shown in figure 4 (*a.e.*). The epithelium is ciliated and possesses many mucous glands, and there is an investment of circular and longitudinal muscle. Immediately posterior to the salivary glands it bends sharply back, both arms of the loop lying in the same horizontal plane (figs. 4 and 5, *l.*). The esophagus then turns back obliquely upward to open ventrally, somewhat to the left of the middle line, into the contractile esophageal pouch (fig. 4, *e.p.*).

Bergh (1876) failed to observe this loop, which apparently is absent in species of allied genera which have been examined, namely in *Elysia, Caliphylla,* and *Hermaea*. Its structure, as reconstructed from transverse sections, is shown in figure 6. This reveals that it is not produced as a result of simple bending, since the region of the bend is extended into a small caecumlike projection with a relatively wide lumen, the division into two being brought about by the sinking in of the dorsal wall. Possible explanations for the presence of both the loop and the caecumlike extension will be discussed later.

The esophageal pouch in *Tridachia* was originally described by Bergh (1876) as the stomach. It was correctly identified in *Elysia viridis* by Pelseneer (1894), and similar structures have been described in *Caliphylla mediterranea* by Brüel (1904) and in *Hermaea dendritica* by Fretter (1940), although she states that this structure is absent in the allied *Limapontia capitata*. In *T. crispata* it is rounded but has a relatively small internal cavity, owing to the great development of muscle in its walls. The epithelium is surrounded first by a layer of circular muscle (fig. 5, *c.m.*) and this in turn by a very thick layer of radiating muscle (*r.m.*). The epithelium is thrown into folds owing to the varying height of its constituent cells. It is ciliated, but there are few mucous glands, although Fretter (1940) identified both these and occasional glands of a second type, possibly digestive, in the esophageal pouch of *E. viridis*. The gastroesophageal ganglia (fig. 7) are closely applied to, indeed partially embedded in, the walls of the pouch and are presumably concerned with the contractions of this organ.

The posterior portion of the esophagus consists of a short, straight tube which leaves the pouch posteriorly in the mid-line ventrally and passes to the stomach. It has a very well-developed investment of circular muscle (fig. 5, *sp.*) which probably corresponds to the sphincter guarding the entrance to the stomach in *E. viridis* (Fretter, 1940). The stomach (fig. 4, *s.*), which was described by Bergh (1876) as the intestine, consists of a small oval organ which merges gradually into the dorsally extending intestine (fig. 4, *i.*). The internal walls of the stomach are thrown into a series of folds so high that they almost obliterate the lumen. The cells are ciliated, but mucous glands are absent.

On either side of the opening of the esophagus are the wide apertures of the main ducts of the digestive diverticula. These ducts have the same general histological character as the rest of the gut, but there are no mucous glands and little surrounding muscle. Fine ducts ramify along the sides of the body and unite to form large ones, all eventually merging into four main ducts, an anterior and a posterior on each side, the posterior being the larger. The two main ducts on each side usually unite shortly before they enter the stomach, as described and figured by Bergh (1876), but conditions vary somewhat in different specimens.

The tubules of the digestive diverticula, instead of being scattered through the body generally as in *E. viridis,* are largely congregated in the thin body folds, although, as shown in figure 8, they do not extend into the ultimate margins. They communicate with ducts which run along the base of these folds (fig. 8, *d.*). The tubules are divided into three regions both topographically and histologically. The proximal regions ramify throughout the middle of the tissues in the basal and middle regions of the folds (fig. 8, *a.d.*). The great majority of the cells in this region are relatively large and contain inclusions of varying size and character. Material fixed in Flemming's fluid indicates the presence of much fatty material. They have all the characteristics of cells concerned with absorption and intracellular digestion, the inclusions representing ingested food material and also the indigestible remnants of intracellular digestion which will later be expelled. By feeding with iron saccharate, Fretter (1940) has been able to demonstrate that similar cells in *E. viridis* are absorptive. Among these cells are a few secretory cells identical with those present in the third region.

The second region of the tubules consists of narrow ducts (fig. 8, *i.d.*) which extend peripherally on either side of the body fold. They communicate with the third region, which consists exclusively of cells filled with great numbers of minute spherules which stain with iron haematoxylin. They are in all probability secretory cells and their contents zymogen granules. These terminal regions of the tubules are always situated immediately below the epithelium (fig. 8, *s.d.*), constituting what Bergh (1876) described as the subcutaneous liver follicles. Between them lie the numerous subepithelial mucous glands (*m.g.*).

There is no muscle around any portion of the tubules and no evidence of ciliation in fixed material, although this does not necessarily imply lack of cilia in life (cf. conditions in the digestive diverticula of the Lamellibranchia, Yonge, 1926). In *E. viridis* there is no such division of the tubules into three regions, the various types of cell being intermingled and the tubules ramifying through the body generally (Fretter, 1940). This localization of secretory and absorptive cells in the tubules of *Tridachia* somewhat resembles conditions in the aeolids (Graham, 1938), but this is due to convergence (a result, probably, of the form of the body) and is no indication of the close relationship postulated by Pelseneer (1894, 1906).

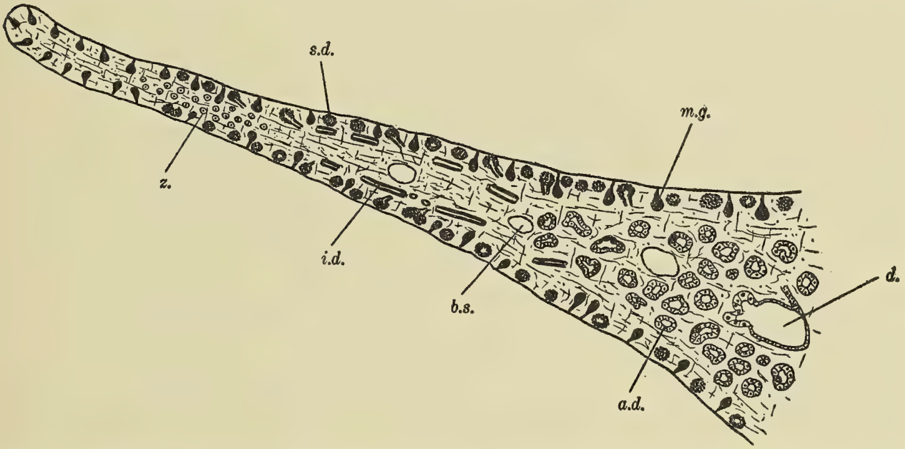

Fig. 8. *Tridachia crispata*, transverse section through the extended body fold. × 45. *a.d.*, absorbing region of tubules of digestive diverticula; *b.s.*, blood sinus; *d.*, duct of digestive diverticula; *i.d.*, intermediate (communicating) region of tubules of digestive diverticula; *m.g.*, mucous glands; *s.d.*, secretory (terminal) region of tubules of digestive diverticula; *z.*, zooxanthellae.

Fretter (1940) identified a third type of cell in tubules of *E. viridis*. This contained lime spherules in life, although these disappear after fixation. Similar cells are present in the aeolids (Graham, 1938). They are believed to be concerned with the regulation of the pH in this region of the gut. The cells in *Tridachia* were not examined in life, so that it is impossible to be certain as to their presence; the assumption is that they probably occur.

To summarize these statements about the digestive diverticula: The ciliated ducts which leave the stomach subdivide into smaller ducts which eventually communicate with the tubules. These are first composed very largely of absorptive cells and are relatively wide in cross section; they merge into narrow ducts which communicate with the terminal portions of the tubules, which lie immediately below the epithelium and are composed exclusively of secretory cells. Some lime cells may be present also.

The intestine (fig. 4, *i.*) runs directly from the stomach dorsally toward the anal opening and is indistinguishable, except in width of lumen, from the stomach. The rectum (*r.*) is a narrow tube with folded internal walls and with very little surrounding muscle, but ciliated, although without mucous or other gland cells. It opens to the exterior at the anus (figs. 1 and 4, *a.*) behind the right tentacle. There is a small sphincter muscle around the opening.

Feeding and Physiology of the Gut

Although the process of feeding in *T. crispata* was not observed, its feeding mechanism is essentially similar to that of *E. viridis* and observations on this animal agree entirely with those of Fretter (1940). The oral cavity is capable of great distension and during feeding is closely applied to the surface of the green algae on which the elysioids feed. Fretter (1940) states that a firm attachment is secured in *E. viridis* by the secretion of both buccal and pedal glands. Owing to its restricted attachment to the adjacent tissues, the buccal mass with the contained odontophore can be extruded through the dilated oral tube so that the exposed teeth on the radula bear against the food. The arrangement of the teeth is such that they function only when, by the action of the buccal retractors, they are withdrawn. Their backward-directed points and serrated posterior surfaces then lacerate the surface of the algae. At the same time the dilation of the lumen of the buccal cavity by contraction of the thick layer of radiating muscles draws in the fluid contents of the punctured cells. The cilia lining the oral cavity and tube assist in this. The whole mechanism is thus adapted for puncturing of plant cells and then sucking out their contents. The radula teeth, although they must be large for this purpose, can seldom if ever suffer any significant abrasion. This probably explains the presence of the ascus, in which the discarded, but still intact, teeth are stored. An essentially similar feeding habit has been described in *Hermaea* and *Caliphylla* by Brüel (1904) and is probably also present in *Limapontia* (Fretter, 1940). The various groups comprising the Opisthobranchia are all highly adapted for feeding exclusively on certain types of food. The Elysiidae are an excellent example of such specialization.

Within the buccal cavity the food is mixed with the secretion, presumably digestive, from the salivary glands. This is probably forced into the lumen by contractions of the vesicles near the terminations of the ducts (Fretter, 1940). Within the esophagus food is presumably conveyed back by the combined action of cilia and muscle. The esophageal pouch is not necessary for this, and the absence in this organ of cells producing a digestive secretion disposes of the suggestion of Brüel (1904) that its contractions in *Caliphylla* force such secretions into the esophagus and stomach. When the pouch dilates, owing to the contraction of the radiating muscles, fluid is drawn in from the anterior esophagus, the sphincter around the posterior esophagus probably preventing the back flow of fluid from the stomach. When the pouch contracts, by the action of the circular muscle, fluid is forced into the stomach. The caecumlike projection at the posterior end of the esophageal loop probably acts in this instance as a valve preventing regurgitation. The presence of the loop, which is absent in all other elysioids so far described, is probably correlated with the greater length of the oral region in *Tridachia*. This involves greater extension of the buccal mass when the latter is extruded, and the presence of the loop permits stretching of the esophagus during feeding.

The need for the pumping action of the pouch would seem to lie in the flattened nature of the body and the consequent great length of the narrow, ramifying ducts of the digestive diverticula. By this means food is forced through these into the ultimate branches. Brüel (1904) suggested this as one function of the pouch in *Caliphylla,* and Fretter (1940) comes to the same conclusion for *Elysia* and *Hermaea*. Its absence in *Limapontia* may be due to the more rounded, compact body, just as its exceptional size in *T. crispata* may be attributed to the extreme flattening and lateral extension of the body in this species.

The small stomach has none of the complicated ciliated sorting regions characteristic of the stomach of Lamellibranchia and of many Gastropoda, in which material is directed into the ducts of the digestive diverticula if small, or, if large, into the intestine. The food of elysioids is such that all can be passed into the diverticula. Circulation within the ducts and tubules may be assisted by ciliary action but is probably in the main due to muscular action, the esophageal pouch forcing fluid in, and contractions of the strands of muscle which ramify everywhere through the substances of the body folds forcing it out again. There is certainly constant movement of these folds in life. In this manner material for absorption and intracellular digestion is carried to the proximal regions of the tubules, and secretion from the distal regions and the indigestible residue after intracellular digestion within the cells of the proximal regions will be forced outward.

Cilia in the stomach, intestine, and rectum are apparently the sole agency whereby the small amount of fecal material is carried to the anus. In the great majority of the Lamellibranchia and in those Gastropoda where the anus opens into the mantle cavity, the intestine is coiled, and gland cells are numerous here and especially in the rectum. Both coiling and the presence of glands are associated with the necessity for consolidation of the feces, which would otherwise foul the respiratory chamber (Yonge, 1935*a*). In *Tridachia* and the other elysioids there is no such need, and consequently no coiling of the intestine and a complete absence of glands. Of the genera she studied, Fretter (1940) found glands only in *Hermaea.*

Association with Zooxanthellae

The record of a new instance of symbiosis with unicellular algae in the Mollusca is of interest. The number of such cases is very limited. In the Lamellibranchia only the Tridacnidae live invariably in association with zooxanthellae, the morphology and habits of the animals being fundamentally modified as a result of this (Yonge, 1936). In the Gastropoda existing records indicate that such association is confined to a few Opisthobranchia, namely *Aeolis glauca* (Hecht, 1895), *Doridoeides gardineri* (Eliot and Evans, 1908), *Melibe rangii* (Hornell, 1909), *Phyllirhoë* (Zirpolo, 1923; Fedele, 1926), *Spurilla neapolitana* and *Favorinus albus* (Henneguy, 1925), and *Eolidina* (= *Aeolidiella*) *alderi* (Naville, 1926; Graham, 1938). There are also certain cases of parasitic infection of fresh-water mollusks where the invading algae, in all cases species of *Chlorella,* are found between and never within the cells of the host. Such infestations have been recorded in *Anodonta cygnea* and *Unio pictorum* (Goetsch and Scheuring, 1926) and in *Limnaea peregra* (Boycott, 1926).

Symbiosis with algae has been claimed to occur in *Elysia viridis*. A. and G. de Negri (1876) found chlorophyll in these animals, but this is to be expected in view of the nature of the food. Brandt (1883) isolated small green organisms 2 to 3.5 microns in diameter from their tissues. He claimed they were symbionts like the larger zoochlorellae or zooxanthellae found in other animals, but Buchner (1930) in his review of such symbiosis in animals is rightly critical of Brandt's conclusions. The latter's observations have never been confirmed.

Every specimen collected of *T. crispata* contained large numbers of the spherical brown unicellular algae collectively designated zooxanthellae. As already mentioned, they are invariably localized in a thin band some little distance in from the margin of the body fold, producing there the appearance in life of a brown line, although the color disappears after fixation. Sections of this region of the body reveal that the algae occur in the connective tissue which fills the interior of the folds and that they extend through the terminal region occupied by the exclusively secretory portion of the digestive diverticula and also a little beyond this (fig. 8, *z.*).

The zooxanthellae (fig. 9) attain a diameter of from 9 to 13 microns and have a prominent nucleus (*n.*), which is usually centrally placed, although it may lie a little to one side. Around this are grouped a series of opaque bodies (fig. 9, *ch.*) which appear dark in color after fixation in osmic acid but largely disappear when fixed in Bouin's or Carnoy's fluid. They apparently contain material of a fatty nature and are probably chloroplasts. Unlike the zooxanthellae of corals (Yonge and Nicholls, 1931) and of the Tridacnidae (Yonge, 1936), this form has no obvious pyrenoid. No evidence of the presence of any carbohydrate reserve was found, nor any clear indication of vacuoles, although there is always a clear area around the nucleus. The cell wall can be seen in section but it is very delicate. Even after fixation in Flemming's fluid, which affects the shape of the algae less than any other fixative, the wall appears wrinkled. That of the zooxanthellae of corals, which contains appreciable amounts of cellulose, never loses its smooth outline after fixation (Yonge and Nicholls, 1931).

The algae lie free in the tissues and are never contained within the cells of the animal. This was unexpected because both in corals (Yonge and Nicholls, 1931) and in the Tridacnidae (Yonge, 1936) the zooxanthellae are invariably intracellular. A number of division stages were observed, so there is no doubt that they increase within the tissues. Wandering cells with inclusions of various kinds are abundant in the region occupied by the zooxanthellae, and it is possible that any algae which die are ingested by these cells.

Significance of the Association

The significance of the association between invertebrates and unicellular algae varies widely in the many different groups in which it occurs. Such associations are found, with rare and somewhat doubtful exceptions, in animals which digest, at any rate in part, intracellularly. We may assume that the association originated in this manner in *T. crispata.* The establishment of a permanent association must vary according to whether the animal is carnivorous or herbivorous. In the former case specialization is demanded, particularly of the animal which has to ingest and tolerate plant cells. In the latter case the algae must be specialized to resist the normal course of digestion by the animal. The possible stages by which the establishment of a settled symbiosis proceeded in the two cases has been outlined elsewhere (Yonge, 1935*b*). It was there also suggested that in a few cases animals may have been infected with an alga already specialized for life in another animal. This is undoubtedly true of *Eolidina alderi,* which acquires the zooxanthellae present in the absorptive cells of the digestive diverticula from the actinian *Heliactis bellis,* on which it feeds and from which it also acquires the nematocysts which lodge in the cerata (Naville, 1926; Graham, 1938). It is also possibly true of the Tridacnidae. In *T. crispata,* which is exclusively herbivorous, the same may well be true, although the association here is certainly more highly developed than in *Eolidina,* since the zooxanthellae occur in the tissues and not merely within the absorptive cells. The position of the algae is so constant that it is probable that they are passed from generation to generation by way of the egg or egg case.

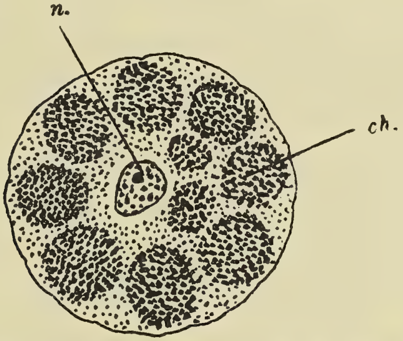

Fig. 9. Zooxanthella from *Tridachia crispata.* × 3000. *ch.,* chloroplast; *n.,* nucleus.

In some cases of this type of symbiosis the animal exploits the algae by consuming them, as in the classic case of *Convoluta roscoffensis* and also in the Tridacnidae, where the zooxanthellae are actually "farmed" in the enlarged mantle edges. In other instances the algae are never consumed; this is the case with those contained in the Madreporaria and probably all Anthozoa (Yonge and Nicholls, 1931). Here the algae possibly play a most important rôle by automatically removing waste products of animal metabolism, notably nitrogenous and phosphoric waste and carbon dioxide. In *T. crispata* there is nothing to indicate that the zooxanthellae are consumed; indeed, they are not sufficiently abundant to provide any important source of food, and moreover the animal possesses highly specialized and thoroughly efficient feeding and digestive mechanisms. But it is possible that the great flattening of the body and its extension into thin body folds laterally and terminally may have affected the efficiency of the circulatory system. It might, therefore, be of survival value if the animals acquired algae which could remove the waste products of metabolism in situ in the mantle folds. Animals with these algae may have proved more efficient and active and so have been selected in preference to those in which excretory matter had to be carried to the relatively distant kidneys.

Summary

1. *Tridachia crispata* is an elysioid opisthobranch with the body extremely flattened dorsoventrally and extended into undulating body folds laterally and terminally. It occurs under stones on the reefs of the Tortugas group and elsewhere in the West Indies.

2. The feeding and digestive systems are described. These have the typical elysioid structure with modifications, notably in the digestive diverticula, correlated with the excessive flattening of the body. Like the other members of the Elysiidae, *T. crispata* is a highly specialized herbivore.

3. Zooxanthellae are habitually present in a restricted zone a short distance from the margin of the body fold. They occur freely within the connective tissue and increase by division. There is no evidence that the animal normally consumes them, but reasons are given for the suggestion that they may be of value to the animal by removing waste products of metabolism produced within the body folds.

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PAPERS FROM TORTUGAS LABORATORY, VOLUME XXXII

XVI

The Ecology and Geologic Rôle of Mangroves in Florida

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With twelve plates and seven text figures

[ISSUED SEPTEMBER 27, 1940]

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The Ecology and Geologic Rôle of Mangroves in Florida

HISTORY AND INTRODUCTION

Mangrove vegetation of the tropical marine littoral has long attracted interest, and has often been mentioned in secular as well as scientific literature. The many inconclusive statements in the literature attracted the writer to the problem of the geologic rôle of mangroves and a thorough study of their ecology.

Bowman (1917), in his excellent review of the early literature on mangroves, cites four classical writers, Theophrastus (305 B.C.), Plutarch (A.D. 70), Pliny (A.D. 77), and Arrian (A.D. 136), who described these trees and took special interest in their ability to grow in sea water. The following summary of the literature to the nineteenth century is a condensation from Bowman (1917).

Theophrastus in his *Historia plantarum* gives the fullest early description of what undoubtedly were *Rhizophora* trees: "These trees are all washed by the sea up to their middle and they are held up by their roots like a polyp, for whenever there is an ebb tide these (the roots) may be seen." His descriptions of the mangroves about the shores of the Persian Gulf could be applied today. The fact that sea water nourished the trees puzzled Pliny the Elder and Plutarch, and some attempts were made by them to explain this "unnatural" condition. These writers probably borrowed from the descriptions by observers on Alexander's march through Asia. Arrian in his *Anabasis* quotes Aristobulus and Nearchus in describing the plants observed on Alexander's march, and the references are essentially all alike. Perhaps Theophrastus summarizes all the observations on *Rhizophora* of his day, and all the later authors copied the accounts of Alexander's companions.

In 1230 the Moorish botanist Abou 'l Abbas en-Nebaty observed mangrove swamps and reported the use of the bark for the tanning of hides. The Moors gave the name "kendela" to *Rhizophora* and "quorm" to *Avicennia*. The modern generic name *Kandelia,* however, applies not to *Rhizophora* but to a related genus of the same family.

Early explorers of the Americas, India, Malay, and the East Indies noted mangrove swamps along the coast and some of them reported the various ways mangroves were employed by the natives. Oviedo (1525) reported that the fruit was used by the natives of the West Indies as a source of food in time of famine. Piso and Marcgraf de Liebstad (1648) described mangroves along the shores of Brazil, and Bauhin (1650) pointed out that in the Malay they were called "mangin" and "bore roots above like stems." Van Rheede (1678) found that in Malabar the bark was used as a cure for diabetes, and later physicians noted the common use of the abundant tannin of the mangroves for burns. Ray (1693) accurately described the unique viviparous seedlings of the mangroves in western India and pointed out that these seedlings are used as food, the wood and bark for tannin, and the roots as a sedative. Plukenet (1669) named mangroves "swamp tree" and first called them "oyster trees" because of the masses of oysters often found attached to the prop roots.

Modern plant geographers have been aware of the uniqueness of this sea-margin forest. Schimper in his *Die indo-malayische Strandflora* (1891) and later in his *Plant-geography upon a physiological basis* (1903) recognized the essential ecological relations of the mangroves to their littoral habitat. He wondered why mangroves did not occur where the inland flora is of a xerophytic type, surmising that their aerial parts could not withstand the excessive dryness of such places. Warming (1925), describing halophytes, gave a good comparison of the floras of the Eastern and Western mangrove regions and noted some of their interesting adaptations. Campbell (1926), Tansley and Chipp (1926), and Raunkiaer (1934) among the plant geographers, and many systematic botanists, as Hooker (1878), Kurz (1887), and Small (1913), have described mangrove vegetation.

It is, however, from the point of view of the modern ecologist and forester that mangroves become important as an ecological problem and economic asset. To the plant ecologist the geologic rôle of mangroves in extending the coast, building up the land, and forming islands is of greatest interest. Some botanists and geologists have described the general ecology of mangrove vegetation in Florida and discussed the geologic rôle of these plants. Their investigations and accounts, though far too brief for such a problem, furnish a good background for the present investigation, and some of their observations and conclusions should be noted.

Curtis (1888) in discussing agencies that have helped build up the peninsula of Florida attributes to the cypresses and mangroves an important rôle. Describing the numerous descending roots of *Rhizophora mangle,* he states: "Floating objects become lodged in these natural weirs, shell fish and other marine creatures multiply in them, and the submerged stems give support to seaweeds and hydroids. In some localities roots become encased with oyster-shells, and this probably is the origin of many oyster bars. The mangrove thickets in the course of time build up a foundation for other species. It is altogether probable that the tree-covered 'islands' in the Everglades and Big Cypress were once mangrove thickets and that present mangrove islands will in time be added to the mainland. As soon as they are elevated above the flow of the tides, the mangroves will give place to species that require only brackish soil, which, in turn, will be replaced by fresh water or inland forms of vegetation." Sargent (1893) adds to the details of this process and speculates further: "For the mass of roots and stems hold a part of the mud washed in by rising tides, and with the aid of the leaves which the mangrove sheds in great numbers every year, gradually consolidate it, and the seedling plants are provided with soil. In this way a vast territory has been formed along the tropical shores of the world, and for a period longer than the human mind can measure the mangrove has been fulfilling its mission on the earth and slowly turns water into land." Pollard (1903) points out that "the actual sea area annually reclaimed by the growth of this remarkable plant would no doubt be surprising if it could be correctly computed. The seeds retain their vitality after long immersion in salt water; and the projecting radicle very soon secures attachment upon a small portion of the reef that has been exposed at low tide. Here then is the beginning of an island." Phillips (1903), Vaughan (1909), Harshberger (1914), Harper (1917), and Gifford (1934) add further details. Their descriptions were nearly all written without either a thorough study of the general ecology of mangrove vegetation or enough field and experimental work to verify their conclusions about the land-building rôle of mangroves.

Other regions have also been studied recently. Freyberg (1930) in Brazil does not attribute to mangroves a very significant rôle in land building. In the Old World, Karsten (1891), Brown and Fischer (1920), Stamp (1925), Watson (1928), and many other ecologists and foresters have made detailed studies of the mangrove forests. The foresters have established definite rules of silvicultural practice for mangrove swamps. Near the teeming populations of the Orient these trees are of great economic importance as sources of firewood, coke, tannin, and other products. Similar studies of their utilization and forest management might well be promoted for the mangrove regions of the Americas.

Plant morphologists and physiologists have not neglected this excellent material. Of their many contributions, those by Göbel (1889), Schenck (1889), Haberlandt (1914), Bowman (1914, 1917), and Cook (1917) are representative. The mangrove's efficient mode of dispersal has attracted the attention of many persons, and the studies of plant dispersion by Guppy (1906, 1917) and Ridley (1930) have included some interesting details of the migrations of these plants.

PURPOSE OF THE STUDY

With the above facts, and more, in mind, this long-term investigation of the ecology of mangrove vegetation and its geologic rôle was begun. Florida was chosen as the first place of study because of its nearness, but the plan is to extend the investigation to other regions as opportunity permits. Nearly all the past studies have been incomplete, particularly on the geologic side. This investigation will attempt to include many phases of the ecology of the mangrove swamps, some phases of the ecology of associated organisms, the migration and establishment of the plants, the nature of the soils, marine factors of the habitat, and the soil accretions, with accurate determinations of coastal and insular changes. Other aspects of the problem will be stressed as they become important during the progress of the work. At present the special purpose is to obtain more accurate information concerning the part mangroves play in land-area changes, soil building, and protection of the coast from erosion.

After five summers and one winter month of field observations supplemented by observations during all seasons by trained assistants, and after some laboratory analyses of habitat factors, studies of growth of the plants under experimental conditions, and other sets of observations, it is evident that a comprehensive or nearly complete report cannot be made for some years. A long series of observations upon the soil accretions, land-area changes, and migration and establishment of the plants is needed. Enough has been learned, however, to merit this preliminary paper, which will indicate the direction the investigation is taking and show some of the most interesting and significant results.

In addition to the primary purpose of making a thorough ecological study and interpreting the geologic rôle of mangroves, it has become obvious that many features of the physiology and morphology of these plants should be investigated. Some work has been done on these, and some information borrowed from others and applied in this study. A great deal more needs to be learned, especially about growth form, manner of root development, and adaptiveness to different degrees of salinity, types of soil, and other environmental factors. It is probable that some of the observations and conclusions here are faulty because of the lack of adequate physiological and morphological information. Even the taxonomy of the species of the Rhizophoraceae and other families to which mangroves belong is in dispute.

ACKNOWLEDGMENTS

Preliminary surveys of southern Florida were made in 1933 and 1934, and more detailed work was begun in 1936 under a grant from the National Research Council. In 1937 the American Philosophical Society also granted aid from the Penrose Fund (J. H. Davis, 1938*b*), and the Carnegie Institution of Washington provided the facilities of the Tortugas Laboratory. Both the latter two organizations aided in 1938. The U. S. Coast and Geodetic Survey helped in many ways by maps, advice, and some transport in the field. Dr. H. S. Wolfe, of the University of Florida Subtropical Experiment Station at Homestead, generously furnished laboratory space and facilities. Dr. O. C. Bryan, of the University of Florida, and the Florida state chemist, Mr. J. J. Taylor, performed many soil analyses. Dr. Herman Gunter, the Florida state geologist, and Mr. Ernest F. Coe, chairman of the Everglades National Park Association, loaned equipment and furnished assistance and advice. The staff and investigators at the Tortugas Laboratory were helpful, and Southwestern College, Memphis, Tennessee, provided supplies and laboratory space. Special credit is due Messrs. Frank M. Ryll and Roy Matthews for their aid as technical assistants in the field and laboratory in Florida. A number of other individuals and organizations aided in the work. To all these I wish to express my sincere appreciation.

SCOPE AND PROCEDURE

A fairly thorough reconnaissance of most of the Florida coasts south of 30° north latitude was made, and regions for special study were chosen. Exploration was by boat, automobile, airplane, and on foot, and was supplemented by examination of air photos, maps, and charts. From the air the zonation of the swamps and depth of the water were most plainly visible. No large area was neglected, so that the results are representative of conditions along all of the Florida coasts.

The regions for study, and particularly the stations for special study, were chosen with regard to the types of mangrove communities and the evidences of land-area changes. Some of the stations were selected at random so as to avoid the error of choosing too many with similar vegetation and environmental conditions. For convenience the Florida coasts were divided into five regions, fairly distinct from one another in many respects; see figure 1. Within each region stations were selected which would be representative of conditions throughout the whole region.

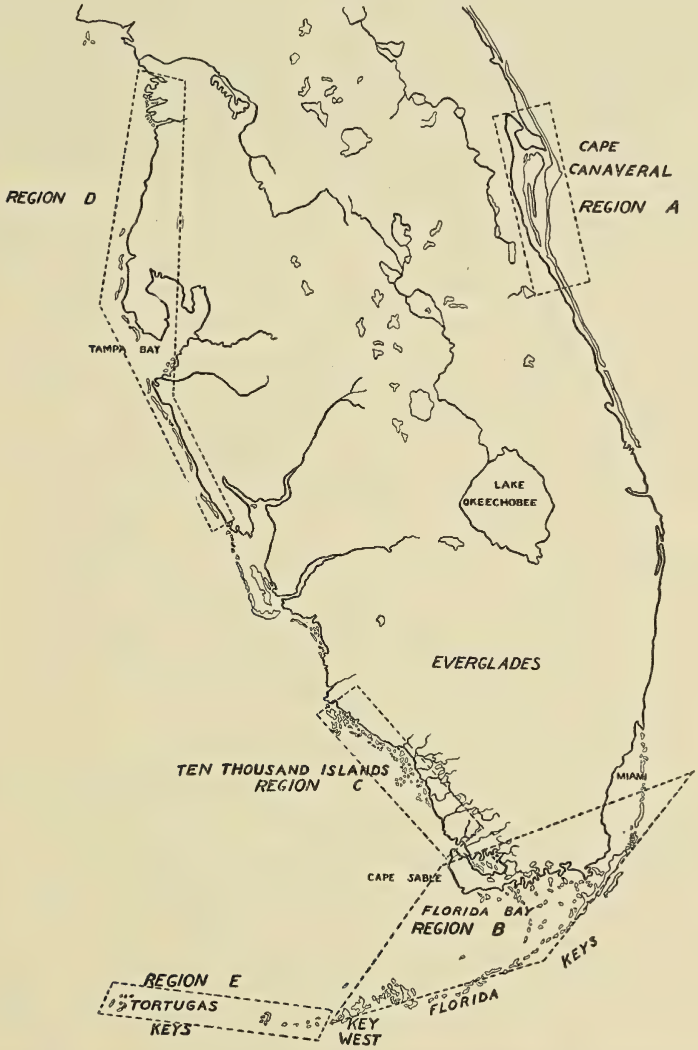

Fig. 1. Map of part of the peninsula of Florida, showing the location of five regions where stations were established for the study of mangrove vegetation and land-area changes.

Region A is located along the central portion of the east coast of Florida and includes the northern portion of the Indian River, Merritt Island, the Banana River, and adjacent territory. Most notable is a group of small islands west from Cocoa Beach, named the Thousand Islands. The soils of this region are composed of fine silicon sand. Salt marshes are more abundant than mangrove swamps. Where mangroves occur they are widely spaced over the salt marshes, or are merely narrow fringes along the estuaries. No mangroves occur along the shores of the Atlantic Ocean. *Avicennia nitida,* the black mangrove, and *Laguncularia racemosa,* the white mangrove, form most of the low thickets. There are only a few specimens of the red mangrove, *Rhizophora mangle,* although it is found farther north on the Gulf of Mexico coast. Residents of this region reported that the mangrove vegetation was much greater before the 1894–1895 freezes, which killed most of the trees. There is no evidence that recent land increases have occurred here, but a thorough study of the plant communities was necessary to determine the conditions of growth on sandy soils and at this latitude, about 28° 20′ north. Scattered mangrove areas occur south of this region along the Indian River, but repeated dredging of the river has altered natural conditions too much for reliable observations on land changes.

The second region (fig. 1, B) is large and very significant. It includes the coastal areas from southern Biscayne Bay around the coast to Cape Sable and extends out over the Florida Keys to Key West. The islands here are of two types: the outer chain of the elongated Florida Keys on well-elevated coral and lime rock, and the inner, smaller keys which dot the shallow bays and sounds behind the outer keys. Most of the Florida Keys were studied southwestward along the entire chain of islands. Many of the inner islands were visited, and some of them proved to be the most significant locations found for studies of changes in soil level and land area.

The northeastern Florida Keys are of sufficient elevation to support a tropical forest vegetation, and some of them slope so steeply to the Straits of Florida that little or no mangrove fringe occurs. In contrast with the outer shores, the sides toward the shoal waters of the bays and sounds support extensive mangrove swamps, and there are areas of appreciable land increase in recent years. The shoal waters between the outer keys and the mainland are divided by cross chains of islands into a number of basins connected by narrow channels. Many islands of different elevations above mean low tide are scattered about in the bays and sounds, and on them the mangroves flourish. The beadlike chains formed by some of the islands may indicate the manner in which some of the elongated islands originated, by the coalescing of a number of smaller islands. Marine sediments are rapidly filling in these shallow-water basins, as indicated by the numerous shoals formed, or increased in elevation, since former surveys. The mangroves that develop in these shoals hasten the sedimentation, forming incipient islands, and organic débris accumulates in the swamps. There is good evidence that a number of the islands and parts of the coast have enlarged in recent years.

The third region (fig. 1, C) is the greatest of all mangrove swamp forests in Florida (Gifford, 1934) and one of the greatest in the world. It extends from the Shark River–Whitewater Bay region to Cape Romano and includes the Ten Thousand Islands and the many river swamps of the southwestern Everglades region. The rivers that drain this part of the Everglades are lined 5 or 6 miles inland by tall mangrove forest, which also covers many of the areas between the rivers. High tides cover most of this low region, and the rivers are joined by back bays to form a labyrinth of watercourses (pl. 1). This great swamp grows on deep peat soils that indicate a long accumulation of organic materials.

The outer islands, called the Ten Thousand Islands, are frequently underlain by shell materials. Nearly all of them are covered by mangroves. Beaches and strand vegetation occur on a few of the higher islands, and some of them are sufficiently elevated to support a hammock forest vegetation.

There is less evidence of land-area changes here than in region B. The charts and maps for this region are not very accurate, and it is difficult to make comparisons of old and recent ones, for fewer surveys have been made. The swamp vegetation, however, is the optimum growth of mangroves in Florida. The mangrove trees are in many cases over 80 feet tall and 7 feet in circumference and are straight-boled and widely spaced as in a typical mature upland forest. At present some parts of the region are difficult to reach, and little botanical work has been done here.

The fourth region (fig. 1, D) extends along the west coast of Florida from Lemon Bay to Crystal River. It includes both the chain of islands along the coast and some parts of the mainland coast. The region is similar to region A, along the Atlantic coast, for the soils of both are dominantly sandy. Region D, however, has oyster and other shell shoals and more calcareous soils than region A, and it also has more mangrove swamps. Some of the islands of this fourth region appear to be of recent origin or enlarging. Salt marshes increase in extent northward, until only a few definite mangrove communities are found in the Crystal River area. North of this region *Rhizophora* has been reported on the Cedar Keys, and some *Avicennia* bushes are found along the Gulf of Mexico coast to Louisiana (Penfound and Hathoway, 1938).

The continuation of the Florida Keys to the islands of the Tortugas group has sometimes been called the Sand Keys (Millspaugh, 1907), and this chain of islands comprises the fifth region of this investigation (fig. 1, E). It extends with few wide gaps to the Marquesas Keys, which form an atoll, and about 40 miles beyond these is a small atoll of calcareous sand islands known as the Dry Tortugas Keys. These low islands are very interesting because their botanical features were described by Millspaugh (1907), and those of the Tortugas group especially by Bowman (1918), so that a good comparison between present and past vegetation can be made. Most of the islands of the Marquesas and adjacent keys are mangrove-covered, and many comparisons and other data show that these areas have changed somewhat.

At the Carnegie Institution of Washington Laboratory on Loggerhead Key of the Dry Tortugas a number of observations were begun in 1937 and 1938. Studies of the migration of mangrove seedlings were made and changes in the vegetation and land areas of some of the keys noted. Over 4000 *Rhizophora* seedlings were planted on Long Key of the Tortugas group to see if a colony of mangroves could be started in this exposed situation and eventually grow into a swamp with certain effects on the soil level and land area. Future observations and experiments in region E should prove most informing, as the ocean currents from Key West toward the Tortugas are very favorable for the study of the dispersal of mangroves.

At the stations established in each region, observations of the vegetation were made and data on soil, water, and other features were obtained. Over 100 localities were selected, probably representing all conditions of mangrove growth in the state. On pages 315–318 is a partial list of these stations with brief notations about the vegetation, soil, plantings, and other matters of interest in this investigation. The environmental conditions at each station and at some places between them were studied. Marine currents, tides, and other factors that affect accretion of soil and dispersion of mangroves were studied in the open sea.

The thousand and more square miles of mangrove areas in Florida are too vast to reach and investigate completely, but this preliminary survey has been extensive enough to be representative of all conditions, and at many localities it has been intensive enough to produce satisfactory results to date. Fortunately the season of field work varied so much climatically that the data obtained and observations made are indicative of both usual and unusual conditions. Even a small tropical hurricane obliged by causing abnormal but significant conditions during part of the 1936 season.

The land-building rôle of mangroves is influenced by many marine factors of a complex nature, and certain studies of oceanography and geology were necessary to understand these factors. Such things as tidal and other marine currents, turbulence of the water, and changes in sea level were difficult to measure or estimate. Sedimentation and accumulation of organic materials could be calculated only in the roughest fashion in so short a time. All these factors can be partly understood, although the observations and data are not of much quantitative value.

The plant populations of the communities were analyzed, although minute statistical methods were difficult to employ in the flooded swamps. Definite zonation into subcommunities is nearly always evident, in many places indicating progressive changes as the soil builds up to higher levels. The associated types of vegetation were studied because the halophytic swamps are not distinctly separated from them, and also because the non-halophytic vegetation is frequently the climax stage of the halophytic succession.

In addition to observations on the plant communities and the factors of their environment, the conditions of seedling and seed dispersal of the mangroves and some associated plants were studied. The establishment and rate of growth of the plants at different localities were investigated, and certain plantings were made in the field and laboratory. Definite areas were marked and measured to obtain data on the extent and rate of soil accumulation, changes in the soil level, and changes in the vegetation over a long period of time. In the laboratories in Florida and in Memphis, Tennessee, plants have been raised to test their growth in different salinities, to make measurements of their development, and to study certain phases of their physiology.

Since this paper treats of two distinct aspects of mangrove vegetation in Florida, it is divided into two parts. Part I considers the ecology of mangroves, and part II the geologic rôle of mangroves. In each part are some observations and conclusions concerned with the other part, for the interpretation of the ecology and that of the geologic rôle of mangroves are related in many ways. The former is considered first, since understanding of the ecologic factors is a necessary background for the geologic study.

Partial List of Stations, with Notations on Location and Vegetation

Key number
of station

Region A

MI 2. Banana Creek, north end of Merritt Island, east of False Cape. Salt marsh with Laguncularia consocies.

MI 3. Big Island, outer one of the Thousand Islands on the Banana River. Salt marsh with scattered groups of *Avicennia* and *Laguncularia.*

MI 5. Near Oceanus, east side of Indian River. Few groups of mature *Rhizophora* on shell and sand soil.

East Coast between Regions A and B

EC 2. Both sides of Indian River at bridge across to Hobe Sound. Three zones of vegetation: (1) river-margin *Rhizophora* and *Avicennia;* (2) salt-water marsh with small *Avicennia* and *Laguncularia;* (3) tall *Laguncularia* with *Acrostichum* on peat and sand soil.

EC 5. Three miles north of Fort Lauderdale, Middle River. River-margin mangroves of all three genera.

Region B

Biscayne Bay

BB 1. East end of Maury Street Canal, Homestead. Two zones: (1) outer marginal mangroves of eroding shore; (2) inner broad zone of dwarf *Rhizophora* in brackish marsh.

CC 1. Caesar's Creek, Bay side, the Rubicon Keys. Mature Rhizophora consocies.

CC 3. Caesar's Creek, Straits side, Christmas Point of Elliott's Key. Mature Rhizophora consocies on 7 feet of peat over coral rock.

Key Largo and Adjacent Waters (see fig. 6)

KL 1. Straits side of Key Largo, Section 19, Township 51, Range 41. 600-yard-wide zone of nearly pure mature Rhizophora consocies on deep peat over coral rock.

KL 2. Garden Cove. Eroding shore. Mixed stand of shore-margin mangroves of all three genera. Plantings.

KL 3. Area north of canal along road from Pelican Roost to upland of Key Largo, toward Cormorant Point. A large area with mature Rhizophora consocies, Avicennia salt-marsh associes, and scrub mangrove facies, on deep interbedded peat and marl soil. Plantings and experimental plots for study of natural survival.

KL 4. Surprise Lake. Scrub mangrove facies on peat. Planting.

KL 5. East side of Jewfish Creek. Marginal fringe of *Rhizophora;* interior Avicennia salt-marsh associes on deep peat.

KL 6. North end of Jewfish Creek, Barnes Sound. Pioneer Rhizophora colony extending out over marl mud covered by marine aquatics. Plantings.

BS 1. Shoal between Division Point and Rookery Key, Barnes Sound. Pioneer Rhizophora colony. Experimental plot.

BS 2. Cormorant Keys. Twenty-year-old mature Rhizophora consocies.

BS 3. Flat Point. Eroding shore. Avicennia salt-marsh associes.

Florida Bay (see figs. 6, 7)

FB 1. Boggies Pass between Blackwater Sound and Florida Bay. Pioneer Rhizophora colony and mature Rhizophora consocies on marl.

FB 3. Porjoe Key and shoals. Recently formed island, mostly flooded. Pioneer Rhizophora colony, mature Rhizophora consocies; in center, some *Avicennia* and *Laguncularia.* Planting.

FB 5. Tern Keys south of Cocoa Point. One island recently formed. All stages of Rhizophora communities on marl.

FB 6. Cocoa Point region. A large area including eroding and depositing shores. Many types of mangrove communities, tropical hammock plants, strand plants.

FB 8. Shore of Mud Bight. Hammocks of tropical hardwoods and palms, no mangrove fringe as shore is being eroded.

FB 15. Shoal northwest of Captain Key. Large pioneer Rhizophora colony.

FB 16. Little Bottle Point Key. Shell material shoal. Mature Rhizophora consocies, strand plants.

FB 17. Little Lowe Key. Interior a fine Avicennia salt-marsh associes with abundant key grass.

FB 18–32. Islands and shoals in Florida Bay. Some areas eroding, others building. Many pioneer colonies; Rhizophora and Avicennia communities common; some areas with enough high soil to support incipient hammocks (fig. 7).

Florida Keys from Key Largo to Key West

KW 1. Grassy Key. Mangrove flats of scrubby scattered mangrove bushes in salt marsh composed mainly of key grass.

KW 3. Parts of Big Pine Key. Mangrove flats on thin marl soil over lime rock.

KW 5. Saddlebunch Keys. Mangrove flats on thin marl soil; Conocarpus transition associes and strand hammock.

KW 8. Tide basins around Key West Roosevelt Boulevard. Rhizophora communities.

Cape Sable

CS 1. Sixteen miles southwest of Royal Palm Park. Fresh-water marsh with dwarf *Rhizophora;* palm and hardwood hammocks. Plantings.

CS 3. North shore of West Lake. Aquatic vegetation of the lake; Rhizophora consocies, buttonwoods, hammocks, Everglades marshes.

CS 4. Coot Bay and south shore of Whitewater Bay. Mangrove fringe forest, open marl prairies, hammocks.

CS 5. Devastated areas along road to Flamingo, results of 1935 hurricane. Plots for study of regrowth of mangroves.

CS 8. Murray Key. High marl areas on island. Avicennia salt-marsh associes.

CS 10. Mid Cape. Beach, dunes, and depressions back of dunes with mangroves. Strand vegetation on beach and dunes.

Region C

Estuaries

SR 1–4. From the mouth of Shark River upstream 6 miles. Mature mangrove forest of tall trees on deep peat soil; upstream, increasing frequency of marsh and hammock plants, decreasing size of mangroves.

BR 1–5. From mouth of Broad River upstream 5 miles. Vegetation very similar to that along the Shark River.

CR 1–9. Chatham River, Sunday Bay, Turner River region. Mature mangrove forest similar to Broad and Shark river regions, but trees smaller and peat soils not as deep, more hammock plants.

Ten Thousand Islands

TI 1. Indian Key. High shell island. Fringe of *Rhizophora;* center of strand and hammock plants.

TI 5. Pavillion Key. Outer shore, beach and strand with strand flora and hammock plants. Inner shore, some mangroves in quiet water.

TI 6. Duck Rock Rookery. Mature Rhizophora consocies 30 years old on shell reef.

TI 10. Pelican Rookery near Sand Fly Pass. Mature Rhizophora consocies; form of trees modified by birds' roosting.

TI 16. Key along Sand Fly Pass. Typical growth of mature Rhizophora consocies on shell flats with thin marl mud layer.

Everglades

EV 1. Edge of dredged canal town of Everglades. Fringe of mangroves.

EV 3. Two miles inland along Turner River. Laguncularia consocies.

EV 7–9. Islands of mangroves around depressions in the marsh Everglades.

Region D

Lemon Bay

LB 1. Bird Key Pass between two islands of mature Rhizophora consocies on sand and peat. Planting.

LB 3. North of road to Chadwick Beach. Avicennia salt-marsh associes on peat over sand.

Sarasota Bay Region to Tampa Bay

SB 1.	Sister Keys. Avicennia salt-marsh associes on sand.
SB 3.	Whale Key. Old deeply flooded Avicennia swamp.
SB 9.	Long Boat Key, bay side. Transect of all the mangrove communities on sand, from *Rhizophora* fringe to dune vegetation.
SB 10.	Phillipi Venetian Isles. Mature Rhizophora consocies on oyster-shell bed.
TC 1.	Shore along breakwater, Manatee River side of Terra Ceia Island. Planting.
TC 3.	Head of tidewater on Manatee River. Small *Rhizophora* as inclusions in Juncus salt marsh.

St. Petersburg

SP 1.	East shore of Boca Ceiga Bay. Mangroves on sand flats, some zonation.
SP 3.	Island in Boca Ceiga Bay. Mature Rhizophora consocies on sand.

Tampa Bay to Cedar Keys

PI 1.	Pine Island on the Gulf of Mexico, west of Brooksville. Salt marsh with few scattered mangroves of all three genera.
CB 2.	Crystal Bay and Homosassa Islands. Salt marshes with few mangroves. Some Avicennia and Rhizophora swamps.
CB 4.	Crystal Bay, mouth of the Crystal River. Brackish-water salt marshes with scattered *Rhizophora.*

Region E

SK 2.	Man Key. Mature Rhizophora consocies, narrow transitional zone, strand flora.
SK 5.	Marquesas Keys, Key A. Strand flora on outer shore, mangrove communities on shore of lagoon.
BK 2.	Pond 1 on Bush Key of the Tortugas Keys. *Laguncularia* and *Rhizophora.* Plantings.
LK 2.	Long Key of the Tortugas Keys. Coral ballast reef with part above normal high tide. A few *Avicennia* and *Rhizophora* forming pioneer colonies. Over 4000 seedlings of *Rhizophora* planted.

PART I. THE ECOLOGY OF MANGROVES

INTRODUCTION

The mangrove swamps in Florida are composed of only a few genera of plants, but they form a unique vegetational zone over the marine littoral areas. The term "mangrove" includes both the type of vegetation and the species composing it. It is, however, more an ecological than a taxonomic concept. Mangrove plants are typically adapted to fixation in loose, wet soils, a dominantly saline habitat, and periodic submergence by tides. They exhibit different degrees of vivipary of the fruits and seeds and typical xeromorphic adaptations, and have respiratory roots. Some botanists include only the family Rhizophoraceae among the mangroves, but a number of genera of other families should be included. In Florida only three species, *Rhizophora mangle, Avicennia nitida,* and *Laguncularia racemosa,* are mangroves. A fourth commonly associated species, *Conocarpus erecta,* the buttonwood, is not a mangrove because it does not have enough of the characteristics given above, although it grows in parts of the swamps and forms a transitional zone between the flooded zones and the inland type of vegetation.

Rhizophora mangle, the red mangrove, has characteristic stilt, prop, or buttress roots and bears the most highly developed viviparous fruits. *Avicennia nitida,* the black or honey mangrove, is adapted to loose, flooded soils and has very characteristic breathing organs, the pneumatophore roots, but it has only partial vivipary of the seeds. The white mangrove, *Laguncularia racemosa,* is not as typically a mangrove as the other two and by some persons is given the name white buttonwood. Its vivipary is partial, and pneumatophores are rare on its roots in Florida.

Although the term "mangrove" can be applied to the individual species because of their adaptations, it is most descriptive of the complex of plant communities which are the mangrove swamps. These swamps form distinct vegetational zones of arborescent plants with which a few plants other than mangroves are more or less commonly associated. The swamps are usually tidal or along estuaries; but the term "tidal" must be qualified. All lands below the high-water mark of spring tides may be referred to as tidal whether they are flooded by salt, brackish, or nearly fresh water. Popular conception has made the vegetation of such areas almost synonymous with mangrove forest. The true mangroves may form only a part of the whole tidal zone; on the other hand, they may extend below the lowest tide level or above the highest tide level.

Tansley and Chipp (1926) consider mangrove vegetation "maritime-swamps" composed of natural nonclimax seral communities, and Warming (1925) describes them as "littoral swamp-forest." Watson (1928) and some others are inclined to consider them an association of the tropical rain-forest formation. In the sense of the terms given by Clements (1936) and Weaver and Clements (1938), mangrove vegetation is not a climax forest, nor an association that is part of a plant formation, but it is a number of seral communities arranged in fairly definite zones which form a prisere mostly of the halosere type in salt water and soil. The final stages of this prisere may lead to the development of fresh-water marshes and swamps, or of some upland climax or subclimax forest community. The associes, consocies, facies, locies, and families of this prisere are of varying composition and structure, forming fairly distinct alternations within the swamps. Some swamps are not distinctly zoned, and some of the communities are not definite stages in the sere. It is difficult to fit these units of vegetation into any conventional classification, and it is recognized that terms employed here may be open to criticism and modification. The communities and their successional relations shown in figure 2 and described in the text are, however, fairly well justified from this study.

Although climatically delimited, the mangrove swamps are primarily edaphic forests, for, as pointed out by Tansley and Chipp (1926, p. 241), their ground water is salt or brackish and they are referred to as an "edaphic group." A very broad meaning of the term edaphic should be accepted here, for otherwise some such term as "halic" or "tidal" ought to be used, and this would further confuse the literature. Edaphic forest communities that owe their character primarily to soil conditions are common in the tropics; the fringing forests of rivers, the fresh-water swamps, and the mangrove swamps are examples. Also large areas in the tropics are covered by natural nonclimax (seral) communities. For these reasons, and others that become more apparent below, the seral and edaphic nature of the mangrove swamps should be well recognized.

In the Old World tropics the mangrove forests are made up of some 25 arborescent or frutescent species, most of which are listed by Warming (1925). In the New World and on the west coast of Africa the flora is much poorer but the swamps may be just as well developed. The zones or associes of the swamps are composed of only a few dominant or codominant species, indicating the intimate adjustments of particular species to certain parts of the littoral environment. Watson (1928) and others have thoroughly classified these zones in the Malay Peninsula and shown their relation to height of inundation by tides and degree of salinity. In general the zones are related to the tides, water level, soil, age of the swamp, and erosive and accretive action of the sea, as well as salinity. Foresters and ecologists alike recognize that the vegetation in some of the zones certainly prepares the ground for subsequent types of vegetation. One type may be followed by another until finally a definitely upland, nonsaline type develops. Figure 2 shows the general successional relations of the mangrove and associated types of vegetation in Florida. Such a succession is evident in many parts of the Florida mangrove swamps, but it is not apparent in all; hence some observers have failed to encounter it. The significance of this succession in land-accretion processes is very strikingly apparent and will be considered more fully later.

Another general aspect of the ecology of mangroves is their efficient mode of dispersal and their ease of establishment on littoral areas. Their geographical distribution is in itself an indication of their wide and probably rapid migration. Complete or partial vivipary is common to most species, being best developed in *Rhizophora mangle* among the Florida mangroves. All the mangroves and most of the associated plants in Florida have buoyant seeds or seedlings which are readily carried by currents to remote places. Some of the seedlings are capable of living many months unattached to the substratum even after extreme exposure to buffeting by waves, and in some cases alternate wetting and drying. The net result of this faculty of dispersion is that mangroves are capable of starting a new forest at great distances from their former home. If they are not found growing in any broad, shallow tropical littoral area, their absence can generally be explained by adverse conditions for dispersal. The geographical distribution of mangroves, then, can be used as an index of ocean currents. Guppy (1917) very ably discusses these currents and plant distribution in the West Indies.

The root systems of mangroves are the most evident of their adaptations, and the variations in their different environments are striking. The roots grow for maximum fixation in the many different types of soil and other substrata, and for optimum absorption of water and nutrients. They are also well adapted to conditions of flooding by their development of aerenchymous tissues. This means that their growth forms are markedly correlated with the range of the tides and type and depth of substratum, as well as the degree of crowding in the community. The prop roots of *Rhizophora* are of many forms (pl. 3, fig. 1; pl. 5, fig. 2). Some form a wide circle of successive tiers arching farther and farther out from the trunk if growing where the tidal range is great and the trees are not crowded; but where there is little or no tide, in dense forest, the roots may be very few. In general the offshore side of the tree sends out more extensive roots than the onshore side. Trees in shallow-water basins with thin soil over rock have the widest-spreading prop-root systems. The masses of these roots are densest in young consocies. In *Avicennia* and *Laguncularia,* the long horizontal roots extend out from the tree only a few inches under the soil surface. The pneumatophores of *Avicennia* are abundant in some situations and entirely absent in others. *Laguncularia* rarely produces any pneumatophores in the Florida swamps. These breathing organs are seldom erected above the high-tide level, and their absence in any locality usually indicates an elevation above normal high tide. Moreover, where the soils have washed in and covered the pneumatophores some idea of the accretions may be obtained. In some places this soil consolidates into rock.

The xeromorphic character of the mangrove leaves has been considered by Warming (1925) and Haberlandt (1914) as indicative of their adaptation to a "physiologically dry habitat." There are other striking adaptations to this saline habitat, but the leaves are the most plastic organs and show the clearest correlations with degrees of salinity. The structure of the leaves in relation to different conditions of the water and soil was studied. The water-storage hypodermis and sunken stomates in *Rhizophora* are typical adaptations. There are decided correlations between leaf size and leaf structure, per cent salinity, and type of soil.

Bowman (1917) shows that the transpiration rate increases with increased dilution of sea water. Some of these and other adaptations will be considered more fully later.

MANGROVE AND ASSOCIATED VEGETATION

INTRODUCTION

The vegetational units of mangrove swamps are many because of the variety of species and the zonation, which is due in the main to water level. Although the number of species in the mangrove swamps of the Old World is greater than that in the New World, the types of communities of both floras are very similar. It is probable that Florida has all the mangrove communities common to the Americas, as many different soil, physiographic, marine, and climatic conditions are found in the state.

Not all the low, littoral areas in Florida south of the regions of killing frost have mangrove swamps. Moreover, along much of the coast the swamp zone is so narrow that a graded series of communities is difficult or impossible to distinguish. Nevertheless there are enough broad zones of mangroves with sufficient definiteness of composition and structure to furnish a good basis for recognition of distinct communities. Those described here include most of the mangrove vegetation of the state. The communities are classified in relation to their plant components and their successional relations. It is not difficult to recognize the communities of the main succession, but it is difficult to classify some of the less distinct communities and assign them any rôle in the littoral succession.

The whole complex of vegetation along the coast of Florida includes so many temperate and tropical types, and habitat conditions vary so much, that any division into communities is more or less arbitrary. The present classification, though as natural as can be determined from this study, may be revised with further observations.

Five main mangrove swamp communities are described, and four lesser communities of *Laguncularia,* also the dwarf forms of the other mangroves associated with marsh plants. Since the mangroves cannot be set apart from intimately associated types of vegetation, some of the latter are described. Some of the features of the habitats are noted. A number of possible seres have been recognized, and the most distinct of these are presented in figure 2. A transect of the main succession is given in figure 3. This latter figure shows the usual relations between the main mangrove communities and the water level, and something of the nature of the soils. Tables of the plant populations of the important mangrove communities and some of the associated types of vegetation are given, showing the presence and relative abundance of important species. A scale from 1 to 10 is employed in most instances to show the relative abundance; however, the numeral 1 often indicates less than one-tenth the greatest number of the same species found in another place. In many instances the numeral 1 merely signifies presence of the species without any indication of its relative abundance. To avoid confusion, fractions are not used. In some instances frequency is considered, so that the value given to each species is partly related to the frequency of occurrence of the species in quadrats or strips located at a number of places within the area of the station, or region, indicated. The species are listed in approximate order of their importance in the community, which was determined by abundance, frequency, coverage, and exclusiveness. The nomenclature for algae is from Taylor (1928), for grasses from Hitchcock (1935), and for most of the other plants from Small (1933). The south Florida region has a very mixed flora, with many endemics and unusual combinations of floral elements from the West Indies with those of the southeastern United States.

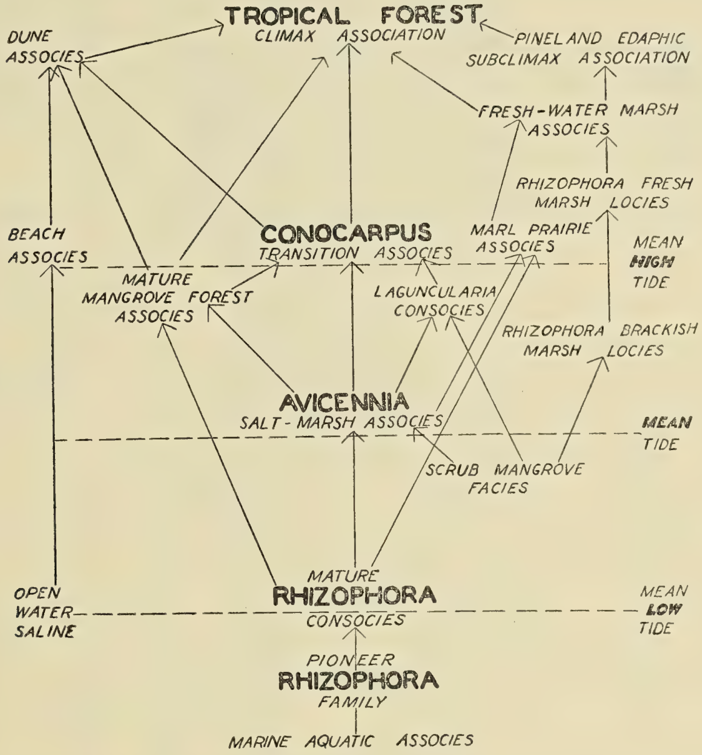

Fig. 2. Successional relations of mangrove communities and some of the associated plant communities. Approximate tide levels are indicated.

The Mangrove Communities

The Pioneer Rhizophora Family

The pioneer community of the mangrove halosere is a family of small red mangroves growing on almost constantly submerged soils (pl. 2). These young

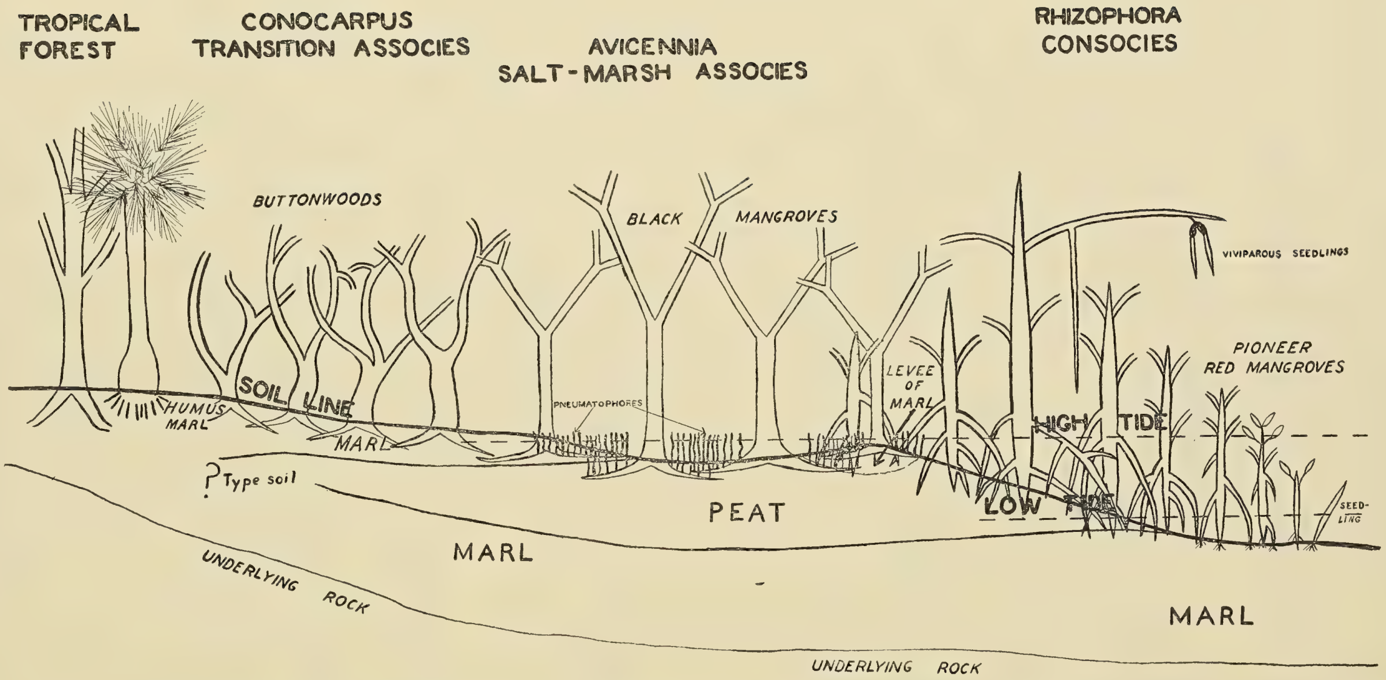

Fig. 3. Diagrammatic transect of the mangrove communities from the pioneer Rhizophora family to the tropical hammock forest, showing approximate tide levels, and soil conditions usually found in a marl soil region.

Rhizophora plants migrate to and become established on shoals, banks, and bars, and in the bays, sounds, and estuaries. They also form the outer fringe of the mature mangrove forest in flooded offshore areas where the water is not too deep or too agitated by waves for the plants to become established. They may become established at a distance from the parent trees because of the ease with which mangrove seeds and seedlings migrate by water (see "Dispersal and Establishment").

The pioneer family starts with *Rhizophora* seedlings that have anchored themselves and begun rapid growth. The young plants soon send out tiers of prop roots, which fix them securely in the loose soils. One- to five-year-old plants compose most of the pioneer stage. Such families often begin on shoals where there is abundant submarine vegetation and marine animals, some of which contribute to their establishment, while others hinder their growth. This ability to form a pioneer community is the most significant feature of *Rhizophora mangle* in the plant succession.

Sedimentation and accumulation of débris increase in rate of deposition among the dense groups of young red mangroves. The soils (sands, marls, and shell fragments) on which the young plants begin are usually the basic soils of the shallow waters. This silting occurs owing to the concerted action of many marine agencies. The presence of marine aquatics facilitates this process. Organic materials increase, and the soil level is built up with growth of the swamp. Once well established, the trees may survive strong wave action, but the pioneer stages do not form where the soils are violently shifted about. The impression given by some writers, as Schimper (1903) and Haberlandt (1914), that species of *Rhizophora* may form an advancing line into the sea along exposed shores is misleading. The mature swamps may front on open bodies of water with strong waves and currents, but they were probably established when these waters were shallower and less disturbed.

Mangroves rarely form in the outer zone, or pioneer stage, of a developing strand vegetation or psammosere. Bowman (1917) and the writer found that the seedlings soon perished on beaches even after planting. They do, however, occasionally contribute to the strand succession, when they begin in sheltered recesses of the coast, or in depressions between dunes.

At most places the typical pioneer Rhizophora family is made up of an average of 20 to 30 young red mangrove plants to a hundred square feet. The associated marine aquatics are mainly the turtle grass, *Thalassia testudinum,* and the manatee grass, *Cymodocea manatorum.* Some of the invertebrates become attached to the plants. *Spartina alterniflora* is abundant in some localities, and in a few places both *Avicennia* and *Laguncularia* seedlings are frequent. At some localities all the mangroves seem to be of the same age, indicating that conditions for their establishment must have been best during one season. In most instances the youngest, smallest plants are in the deepest water, farthest away from the thicker stand of the body of the community. The plants increase in size toward the center of a young swamp (pl. 2), or toward the shore, so that in the case of an island-like colony on a shoal, the outline of the community is dome-shaped. Table 1 gives floristic and quantitative data for this community, and shows some of the floristic changes that occur when a thick swamp forest replaces the pioneer Rhizophora family and a mature Rhizophora consocies is formed.

TABLE 1. *Presence and relative abundance of the most important species of the pioneer Rhizophora family and mature Rhizophora consocies*
(Scale 1 to 10)

SPECIES	REPRESENTATIVE STATIONS					
	KL 6	BS 1	FB 3	FB 22	LB 1	SB 9
PIONEER RHIZOPHORA FAMILY						
Rhizophora mangle 1–3 yrs	8	10	7	10	10	7
Rhizophora mangle 4–8 yrs	8	5	9	5	1	9
Thalassia testudinum	10	10	9	5	10	7
Cymodocea manatorum	3	2	1	....	3	....
Spartina alterniflora	....	....	....	....	3	5
Caulerpa cupressoides	2	1	1	2	....	....
Acetabulum crenulatum	2	3	2	1	....	....
Gracilaria cornea	4	3	3	1	....	....
Halimeda tribens	2	1	4	1	....	....
Sargassum natans	1	1	2	3	1	....
Batophora ovalis	2	....	....	1	....	....
MATURE RHIZOPHORA CONSOCIES						
	KL 1	BS 2	FB 5	TI 6	SB 10	SK 5
Rhizophora mangle	10	10	10	10	10	10
Avicennia nitida	1	1	2	1	....	2
Laguncularia racemosa	4	....	1	1	....	2
Spartina alterniflora	....	....	2	5	1	....
Batis maritima	2	1	2	1	....	....
Salicornia perennis	....	1	1	1	....	....
Dondia linearis	....	....	1	....	1	....
Acetabulum crenulatum	1	3	1	....	....	2
Caulerpa cupressoides	2	1	....	....	....	1
Gracilaria cornea	1	1	1	....	....	1
Batophora ovalis	....	1	2	....	....	....

The Mature Rhizophora Consocies

Normally the small plants of the pioneer Rhizophora community grow into a mature swamp with but few additions of other floral elements. There is usually a definite increase in accumulations of inorganic and organic débris. In about 20 to 30 years the young mangroves become so thickly entangled that their roots act as natural weirs to catch débris. The trees become so well anchored that only the most violent hurricane can disturb them. The plants that now invade this zone of the swamp take advantage of the protection and soils afforded by the thick growth of trees. The marine aquatics decrease in number. The consocies may persist as a nearly pure community of *Rhizophora mangle* for a long time with no evident change (pl. 3, fig. 1); or it may rapidly change by invasion of other species and accretion of soils. This latter change usually brings about the formation of an inner zone of the black mangrove, *Avicennia nitida,* associated with various quantities and kinds of salt-marsh plants.

Over most of the newly formed islands the red mangrove is the only tree, and on some the only seed plant. In such pure consocies it forms one of the unique forests of the world. The great masses of lenticular stilt or buttress roots and the heavy green foliage, together with the twisted and contorted character of many of the trunks, give a striking character to the community. The mud, rock, shell, or peat soils of the forest floor are covered by an interlocking tangle of the branching stilt roots. There are few or no openings in the foliage overhead. At low tide the soil may be exposed, while at high tide many, but seldom all, of the arching roots are flooded. Table 1 gives an idea of the paucity of the flora, and plate 3, figure 1, of the thickness of the growth. Invertebrates, especially coon oysters, *Ostrea frons* (pl. 3, fig. 2), and some of the pulmonate snails live on the roots and stems, but few of the algae or the aquatic seed plants of the shoals persist. Only the abundant mangrove seedlings seem to thrive below the canopy. These grow and adequately replace their elders.

The average size of the trees and the extent and character of their branching root systems vary with habitat conditions, especially with the range of the tide and the nature of the soils. If the tide has a wide vertical range, the stilt roots are farther up the stem than if there is little change in tide levels. The red mangrove trees are profusely branched in the consocies type of forest, especially on marl soils, but their growth form is different in old, mature swamps, where they may be straight-boled and free from lower limbs, as are those along the rivers in the southwest Everglades mature mangrove forest associes. Growth form is also modified by birds, which use some of the trees for roosting and nesting and may cause flattening of the tops and broken limbs. In general, the red mangrove trees of the mature Rhizophora consocies are about 4 to 10 inches in diameter and seldom over 30 feet tall, with dome-shaped crowns. The heavy, dark green foliage forms a thick canopy. The thick, shiny leaves turn yellow and fall off at all seasons, but most abundantly during the summer. Some forests have very few fruiting trees, and in others the trees are loaded with the long, pendent seedlings (pl. 4, fig. 1), which weight the nearly horizontal limbs down toward the water. In some swamps the red mangroves never seem to attain tree size, in others they grow large enough for the timber to be of commercial use. In such cases some of the trees may be nearly 100 feet in height with straight, unbranched trunks.

There are two distinct types of aerial root. Besides the stilt or prop roots, there are flexible drop roots that descend from the branches. They are less common than the larger, stiffer, arching stilt roots, but they can reach the soil or water and branch into feeding roots. Some specimens have large horizontal or inclined trunks that extend almost a hundred feet along the ground, supported by their stilt roots throughout most of their length. In such cases probably the trunk has been blown over by a storm, and the roots have developed to hold up its weight as it grew.

Some growth forms should be more thoroughly studied. The dwarf form of *Rhizophora* is termed "chico" (pl. 4, fig. 2) and the large form "grande" (pl. 5, fig. 2) in most Spanish-speaking countries, but these do not seem to be distinct species as some persons have supposed. Plantings of seedlings from both have been made in the same locality. If they grow up to be of the same form, there will be little doubt of their being the same species.

Rhizophora may dominate this consocies to the complete exclusion of all other mangroves, in which case the community might be considered a Rhizophoretum (Warming, 1925). However, even though other mangrove species are associated, the mature Rhizophora consocies includes all mature communities dominated by *Rhizophora.* Many Rhizophora zones of the swamps are a mixture of all three species of mangrove with only partial dominance of *Rhizophora.* In such cases the red mangroves are usually most abundant in the outer parts of the swamp zone. *Laguncularia* is more often associated with *Rhizophora* than is *Avicennia* in the usually inundated parts of the swamps. Toward the inner part of the Rhizophora consocies *Avicennia* becomes more frequent and finally replaces *Rhizophora* as the dominant plant, thus forming a new zone.

In some places nearly pure stands of mature red mangroves form a definite zone far inland near the uplands or the fresh-water marshes. Such zones are localized along watercourses and around ponds or lakes, where the waters are sometimes only mildly brackish, or even without any easily measurable quantity of salt.

Whatever the cause, the widespread occurrence of *Rhizophora* is very significant. This species is dominant in more mangrove communities than is either of the other two. It is so characteristic in its growth form that some observers consider only the swamps dominated by *Rhizophora* as typical mangrove vegetation.

A mature Rhizophora consocies swamp is marginal along many coasts without any pioneer stage offshore from it. This condition usually indicates no appreciable land extension seaward. As the trees mature they are able to send roots out into the deeper waters beyond the depth at which mangrove seedlings can gain a foothold. Such trees seem to be "walking out into the sea," without benefit of pioneers. Even in such places of deeper water, soils may accumulate among these roots and thus extend the swamp. Along flowing rivers and tidal passes the mangroves are nearly all mature *Rhizophora* trees with boles inclined out toward the streams and outer roots inserted in the deep waters without contact with the soil. In such situations the mangroves advance very slowly if at all into the deeper waters. Even though not advancing into the sea, many of these swamps replace themselves very efficiently, accumulate soil débris, and protect the shores.

A narrow levee-like ridge is sometimes formed along the inner side of the Rhizophora consocies zone, shutting off direct tide and wave action from the Avicennia salt-marsh associes within. Thus vigorous, mature *Rhizophora* trees extend from constantly submerged saline areas up to the high levels of the levees, which are even above mean high tides. The outer Rhizophora zone is almost always inundated by the tides and does not vary as much in its salinity as the more inland Avicennia and Conocarpus communities.

The Avicennia Salt-Marsh Associes

The regularly or only occasionally flooded areas back of the outer Rhizophora consocies are typically covered by a mature growth of black mangroves, with salt-marsh plants covering the floor of the open forest (pl. 5, fig. 1; pl. 6). Such a zone of the swamp is in distinct contrast with the close, tangled growth of the red mangroves. Here *Avicennia nitida* grows to large size, frequently averaging over a foot in diameter. The trees are not very tall, as they are widely spaced. The salt-marsh plants are such succulent shrubs as *Batis maritima* and *Salicornia perennis* and different grasses. Where the water is deep there may be little marsh vegetation, but the tangle of salt-marsh plants with the many pneumatophores of *Avicennia* is most characteristic of this associes. They aid in retarding water currents and thus facilitate sedimentation, and their decaying parts add to the organic débris accumulating in the swamp.

Avicennia has a number of growth forms, for it may be tall and unbranched or grow as dwarfed and gnarled bushes. The radiating roots extend outward from the trunk just a few inches below the soil surface and usually send up erect aerenchymous pneumatophores, which grow upward through as much as a foot of soil and 2 to 3 feet of water. Some of these asparagus-like breathing organs are branched, but few become larger than a half-inch in diameter. In some places they are so thick that they hinder passage through the swamp. The leaves of *Avicennia* are also distinctive, for they are at times covered by salt crystals secreted from the leaves, and for this reason *Avicennia* has been called the "salt tree."

The shallow surface water, if any, in the Avicennia salt-marsh associes zone is not normally thoroughly changed by the tides, and stagnation and consequent increase in salinity may result; on the other hand, rains dilute it, and help cause extreme variations in salinity. This associes is frequently confined to flooded paludal basins surrounded by low natural levees. Some settling of the accumulated soils is evident in such basins, and in many cases the composition and growth form of the trees is such that a reduced form of the Avicennia associes results; this scrub-form community is known as a scrub mangrove facies. The most mature and most characteristic portions of the Avicennia salt-marsh associes, especially on marl soils, are not regularly flooded. The trees are not rapidly replaced by natural growth, and as they die the community becomes more and more open, with an increase in the salt-marsh vegetation or in number of plants from the Conocarpus transition associes, which is frequently adjacent to the inner edge of the black mangrove zone. If the area becomes completely treeless, an open salt-marsh or prairie type of vegetation is formed. Large areas in southern Florida are covered by these marl prairies and marshes, some of which were probably derived from mangrove swamps.

Table 2 gives the important species of the Avicennia salt-marsh associes and of the related scrub mangrove facies. Not only is the flora of this Avicennia salt-marsh associes more varied than that of the Rhizophora communities, but there are many more local variations, owing to the invasion of plants from the higher soil zones of the swamps, or from the associated types of vegetation, or both. *Laguncularia* is in some places abundantly associated with the black mangroves. Although *Batis maritima* and *Salicornia* are most frequent and constant in some

TABLE 2. *Presence and relative abundance of the most important species of the Avicennia salt-marsh associes and the scrub mangrove facies*

(Scale 1 to 10)

SPECIES	AVICENNIA SALT-MARSH ASSOCIES: STATIONS					SCRUB MANGROVE FACIES: STATIONS		
	General	KL 3	KW 7	FB 18	SB 1	KL 3	KW 1	SB 9
Trees and shrubs:								
Avicennia nitida	9	10	8	9	10	1	2	3
Rhizophora mangle	2	2	1	1	1	4	1	3
Laguncularia racemosa	1	1	......	......	1	2	1	2
Conocarpus erecta	1	......	......	1	......	......	1	1
Batis maritima	7	7	9	3	7	1	2	2
Salicornia perennis	3	3	1	4	1	6	5	3
Borrichia frutescens	1	......	......	2	1	1	......	1
Lycium carolinianum	1	1	......	1	1	......	......	1
Bumelia angustifolia	1	......	......	1	......	1	......	1
Ecastophyllum brownei	1	1	......	......	......	......	......	......
Coccolobis uvifera	1	......	......	......	1	......	1	1
Rhabdadenia biflora	1	......	1	......	......	1	......	......
Herbs and ferns:								
Sporobolus virginicus	2	1	2	1	3	......	1	2
Monanthochloë littoralis	2	......	5	7	......	......	4	......
Dondia linearis	1	1	......	......	1	......	1	1
Sesuvium portulacastrum	1	2	1	......	1	......	1	1
Acrostichum aureum	2	......	......	......	1	......	......	......
Spartina alterniflora	1	......	1	......	2	......	......	1
Spartina spartinae	1	......	......	1	......	......	3	......
Spartina cynosuroides	1	......	1	......	......	......	......	......
Juncus roemerianus	1	......	......	......	1	......	......	1
Fimbristylis castanea	1	......	......	1	......	......	1	......
Cyperus ottonis	1	......	......	......	......	......	......	1
Chloris glauca	1	......	......	......	......	......	......	1

places, as on the low islands of Florida Bay and the Florida Keys, the key grass, *Monanthochloë littoralis,* is abundant on marl soils. On sandy soils *Sporobolus virginicus* and some species of *Spartina* are abundant. In a few usually shaded or constantly inundated places few or none of the salt-marsh shrubs and herbs are present.

The Conocarpus Transition Associes

Conocarpus erecta, though not always abundant in this community, is its index species. This associes usually occurs next to the inner edge of the Avicennia salt-marsh associes, and may be independent of either flooded or strongly saline soils. It often extends much farther inland than the mangrove communities and is typical seaward from the tropical hammock forest, strand vegetation, dry pinelands, or fresh-water marshes. In many ways the associes is an ecotone or transition zone between mangrove vegetation and some inland, upland, non-halophytic form of vegetation.

Table 3 gives some idea of the number of tree and shrub species associated

TABLE 3. *Presence and relative abundance of the most important species of the Conocarpus transition associes and the tropical forest climax association*

(Scale 1 to 10)

SPECIES	CONOCARPUS TRANSITION ASSOCIES: STATIONS			TROPICAL FOREST CLIMAX ASSOCIATION: REGIONS	
	FB 6	CS 3	KW 7	Miami to Cape Sable	Florida Keys
Trees and shrubs:					
Conocarpus erecta	6	5	5	1	....
Avicennia nitida	2	1	2	....	....
Laguncularia racemosa	1	1	1	....	....
Rhizophora mangle	1	2	1	1	....
Sabal palmetto	2	3	....	2	1
Ficus aurea	....	1	....	2	1
Coccolobis laurifolia	1	1	1	2	1
Chrysobalanus icaco	1	1	....	1	....
Eugenia buxifolia	1	1	1	1	1
Eugenia axillaris	1	1	....	1	1
Salicornia perennis	2	1	1	....	....
Batis maritima	1	2	1	....	....
Borrichia frutescens	1	....	2	....	1
Lycium carolinianum	1	1	1	....	....
Bumelia angustifolia	....	1	....	1	....
Randia aculeata	1	....	1	1	1
Jacquinia keyensis	1	....	1	1	1
Sophora tomentosa	1	....	1	1	1
Casuarina equisetifolia	1	....	1	1	....
Metopium toxiferum	1	1	....	1	1

(*Continued on following page*)

TABLE 3—*Continued*

SPECIES	CONOCARPUS TRANSITION ASSOCIES: STATIONS			TROPICAL FOREST CLIMAX ASSOCIATION: REGIONS	
	FB 6	CS 3	KW 7	Miami to Cape Sable	Florida Keys
Trees and shrubs—*cont.*:					
Pisonia aculeata	1	1	....	1	1
Drypetes diversifolia	1	....	1	2	2
Ichthyomethia piscipula	1	1	1	2	1
Sideroxylon foetidissimum	1	....	1	3	5
Bursera simaruba	1	....	....	2	3
Swietenia mahagoni	1	....	1	2	2
Krugiodendron ferreum	1	1	....	2	2
Simaruba glauca	1	....	1	2	3
Carica papaya	....	....	....	1	3
Ocotea catesbyana	1	1	1	3	1
Lysiloma bahamensis	....	....	....	2	2
Torrubia longifolia	....	....	....	1	1
Quercus virginiana	....	....	....	2	....
Laurocerasus myrtifolia	....	....	....	2	1
Sapindus saponaria	....	....	1	1	1
Exothea paniculata	....	....	....	1	1
Colubrina colubrina	....	....	....	1	....
Trema floridana	....	1	....	1	1
Erythrina arborea	1	1	....	1	1
Solanum verbascifolium	....	....	....	1	2
Hippomane mancinella	....	2	....	1	....
Bucida buceras	....	....	....	....	2
Terminalia catappa	....	....	....	....	1
Chrysophyllum olivaeforme	1	....	....	1	1
Gossypium hirsutum	1	....	....	1	....
Yucca aloifolia	1	....	....	1	....
Acanthocereus pentagonus	1	....	....	1	....
Cocos nucifera	....	....	1	1	1
Roystonea regia	....	....	....	2	....
Thrinax parviflora	1	....	....	1	....
Thrinax microcarpa	....	....	....	....	1
Paurotis wrightii	....	1	....	1	....
Herbs:					
Sporobolus virginicus	2	1	1	....	....
Monanthochloë littoralis	....	....	4	....	....
Spartina spartinae	1	2	....	....	....
Spartina bakeri	....	1	....	....	....
Fimbristylis castanea	1	....	....	....	....
Juncus roemerianus	....	1	....	....	....
Mariscus jamaicensis	....	3	....	....	....
Dondia linearis	1	1	1	....	....
Sesuvium portulacastrum	1	....	1	....	....
Eupatorium capillifolium	....	1	....	....	....
Solidago leavenworthii	....	1	....	....	....

(*Continued on following page*)

TABLE 3—*Continued*

Species	Conocarpus transition associes: stations			Tropical forest climax association: regions	
	FB 6	CS 3	KW 7	Miami to Cape Sable	Florida Keys
Herbs—*cont.*:					
Flaveria linearis	1				
Pluchea odorata		1			
Melanthera deltoidea	1				
Rivinia humilis			1	1	1
Iresine vermicularis				1	1
Metastelma blodgetti				1	1
Afzelia pectinata					1
Panicum latifolium				1	1
Paspalum ciliatifolium				1	1
Cenchrus carolinianus		2	1	1	
Tricholaena rosea				1	1
Epiphytes, air plants, and orchids:					
Tillandsia recurvata	1		1	1	1
Tillandsia circinata		1		1	
Tillandsia utriculata		1	1	1	1
Tillandsia fasciculata	1		1	1	1
Encyclia tampensis		1		1	1
Ancheilum cochleatum				1	1
Cyclopogon cranichoides				1	1
Cyrtopodium punctatum				1	1
Vanilla articulata		1		1	
Peperomia spathulifolium				1	1
Ferns:					
Acrostichum aureum		1		1	
Adiantum tenerum				1	
Nephrolepsis exaltata				2	1
Dryopteris ampla				2	1
Blechnum serrulatum				1	1
Campyloneuron phyllitidis				1	1
Phlebodium aureum				1	1
Polypodium polypodioides				1	
Tectaria minima				1	
Vittaria lineata				1	

with *Conocarpus* in this transition zone. Many of the tree species are poorly developed, as they occur in this zone simply as migrants from the adjacent forests. The aspect of the community is that of an open stand of trees and shrubs with some salt-marsh plants on the forest floor. In only a few places is there a thick stand of the buttonwood. The cabbage palm, *Sabal palmetto,* the strangler fig, *Ficus aurea,* and the stoppers, *Eugenia,* are some of the associated hammock-forest species. The flora varies greatly from place to place and includes some very localized species. In many areas it is difficult to determine the most constant species.

The soil and water of the Conocarpus transition associes are usually only moderately saline. In most places the soils are not regularly flooded by either fresh water or brackish water, as tides seldom reach as far inland as this zone. Toward the interior, however, *Conocarpus* may thrive in and around depressions filled at least seasonally with deep surface water that contains little or no salt. In other places, as on the salt flats (which will be described later), *Conocarpus* grows in nearly dry soils with a high concentration of salt. Besides being facultative to amount of soil or surface water, *Conocarpus* is a facultative halophyte. Many of the associated species also seem to be facultative halophytes, even though they normally prefer nonsaline situations.

The growth form of *Conocarpus* is frequently sprawling, twisted, and knotty, seldom erect. Its limbs hold many epiphytes on the loose bark. The wood is very good for charcoal and as an insect smudge. The habit of taking root from parts of the stem bent into the soil is of interest because it enables the trees to recover after being bent over by a storm (see Simpson, 1932).

In many instances there is good evidence that the soils of the Conocarpus transition associes were built up by mangrove growth, and the zone is a definite part of the mangrove halosere; but in some situations there is little or no evidence of a succession. The latter is particularly true on the non-flooded sand soils of dunes and along the edge of pinelands. Where heavy, dry marl soils occur it is difficult to find any evidence of underlying mangrove soils. The transition zone may be very narrow, or the mangroves may even be in direct contact with the climax forest without any definite Conocarpus transition associes present.

The Mature Mangrove Forest Associes

The southwest Everglades coast of region C (fig. 1) is covered by a tall, vigorous growth of mangroves of all three American species with the buttonwood, palms, and other upland plants associated in small numbers. Few persons know that such a mature forest community of mangroves exists, and too frequently the smaller growth of mangroves has been described as typical. Such a forest as this is probably the optimum growth of mangroves in the Americas. See plate 5, figure 2.

The tallest trees are red and black mangroves and occur along the lower parts of the estuaries. A narrow zone of smaller *Rhizophora* trees usually fringes the rivers, but back of this thicket fringe is an open forest of large, tall trees, about 60 per cent of which are *Rhizophora,* and about 30 per cent *Avicennia.* The forest is not well zoned into communities like those described above, and with the mangroves are a number of associates. Farther upstream the number of associated tree species increases, the mangroves are smaller, and Everglades marsh plants become more frequent. There are many local variations in the plant populations and general character of the swamps. Most of these observations are based on trips taken up the Shark, Broad, Chatham, and other rivers, which also furnished the data for table 4. Further study of this forest is needed, for it is more nearly like some of the Old World mangrove forests than any other described in the New World.

The tidal rivers that drain the Everglades and these swamps are from 6 to 12 feet deep and form by inland connections a labyrinth of watercourses, with many large bays or lakes a few miles back from the coast. The high spring tides, at some places over 4 feet, push salt water far up the rivers. Great quantities of fresh water are drained off the Everglades down these rivers during the summer wet season. The swamps are deeply and regularly flooded by both these agents, but are less deeply flooded by the normal tides. The flooding waters are seldom strongly saline.

TABLE 4. *Presence and relative abundance of the most important species of the mature mangrove forest associes*
(Scale 1 to 10)

SPECIES	STATIONS		
	BR 1–4	SR 1–4	CR 1–5
Trees and shrubs:			
Rhizophora mangle	5	6	5
Avicennia nitida	3	3	4
Laguncularia racemosa	1	1	2
Conocarpus erecta	2	1	1
Sabal palmetto	1	1	1
Batis maritima	7	6	8
Salicornia perennis	2	1	2
Borrichia frutescens	1	1	2
Lycium carolinianum	1	....	1
Eugenia axillaris	1	1	....
Eugenia buxifolia	1	1	....
Randia aculeata	1	....	1
Bumelia angustifolia	....	1	....
Malache scabra	1	....	....
Coccolobis uvifera	....	....	1
Rhabdadenia biflora	1	....	....
Borrichia arborescens	....	1	....
Ecastophyllum brownei	....	1	....
Herbs and ferns:			
Acrostichum aureum	2	3	1
Spartina alterniflora	1	1	1
Spartina spartinae	1	....	1
Juncus roemerianus	1	1	....
Sporobolus virginicus	1	....	1
Hymenocallis keyensis	2	1	1
Dondia linearis	....	....	1
Tillandsia circinata	....	1	1

Deep peat soils are characteristic. Near the river mouths they are over 10 feet deep in most places, and may be 14 feet deep. Upstream their depth decreases until it is only a few feet near the marsh Everglades. The nature of these organic peat soils and their significance will be discussed later. They indicate a long-term occupation of this region by mangroves, as the peat is largely of mangrove remains. The present trees are able to send feeding roots to great depths. The peat in places is surfaced with mud. In some places the elevated accumulations of marl or shell are covered by buttonwoods, palms, and other upland plants within the broader expanse of the swamp.

It is probable that these swamps have changed very little in many hundred years. There is little evidence of a succession except where the swamps join other types of vegetation. The hammock-forest inclusions are on drier, heavier, higher soils than the mangroves, and may have been islands within the swamps when they began, or may be the results of soil accretions above normal high water. The level of the swamps increases inland, and salinity decreases, so that a gradual change to fresh-water marshes is accomplished. The Lostman's River limestone, a type of the Miami oölite limestone, gradually slopes toward the Gulf beneath the mangrove soils. The wide extent and mixed character of this forest is probably due to the slow accumulation of peat, mud, shells, and other materials over this shelving rock. That the present islands offshore are coalescing with one another and with the main mass of the swamp seems probable. The present rivers may be the remains of former tidal passes between islands.

Whatever the conditions that brought about this unique plant community, and may lead to gradual changes in its composition, the present forest is superb. Some of the red mangroves are over 80 feet tall and 7 feet in circumference. Many of the black mangroves are as tall but are usually more branched. The forest is thick enough to produce straight-boled trees with little underbrush. Natural reproduction on the forest floor is common, but the salt-marsh plants are sparser than in most swamps with *Avicennia*. In many places *Rhizophora* produces few stilt roots or aerial roots, and *Avicennia* few or no pneumatophores. Ferns, spider lilies (*Hymenocallis*), shrubs, buttonwoods, and the cabbage palm (*Sabal palmetto*) are frequent and, with the tall mangroves, give the appearance of an upland mesic forest. The marginal hammocks and hammock inclusions may include such typical palms as the royal palm, *Roystonea regia.*

In figure 2 this swamp was not included as a part of the main succession from open water to upland forest. It may be a significant stage in such a succession, but zonation is less evident than in other mangrove areas, and development toward a climax is less obvious and very slow. This swamp forest may be considered a subclimax type of vegetation.

The Laguncularia Consocies

Laguncularia occurs in all the mangrove communities but is seldom the dominant or significant tree. It grows most abundantly either along the inner margins of the Avicennia salt-marsh associes or as nearly pure consocies near the brackish-water marshes. In the latter situation the water is usually only slightly brackish, and the growth of this mangrove is a thicket stand of slender trees. The white mangrove is also common as stunted trees within the scrub mangrove facies. In some instances it is abundant within the mature Rhizophora consocies. Therefore, no particular zone of the swamps can be assigned to it.

Where it is most abundant, the community is such a pure stand of *Laguncularia* that it forms a consocies. The trees are crowded and there are few or none of the secondary species. In some instances such a community occurs between the Avicennia salt-marsh associes and the Conocarpus transition associes, and may be a part of the mangrove halosere.

In many instances the Laguncularia consocies is flooded with mildly brackish or nearly fresh water 1 to 2 feet deep. Some of the thick stands of slender trees occur along dredged ditches and canals on high soils. Thus it seems that no particular habitat is definitely most suitable for *Laguncularia.*

Dwarf-Form Mangrove Communities

In many situations mangroves fail to reach their normal size, growing as dwarfed trees or bushes and frequently with other characteristics of an abnormal growth form, distinctly different from that found in more favorable situations. These dwarfed forms occur along so much of the coast that some observers have described them as typical. The communities they form and the conditions that produce them are many, but there are several fairly distinct facies and locies. In many cases they are an only slightly aberrant community, and there is not always a sharp line of demarcation between a dwarf-form mangrove community and one of normal growth form. Three more or less distinct types are recognized. The commonest of these is the scrub mangrove facies, with some of all three mangrove species present. The other two communities consist of subdominant dwarfed *Rhizophora* bushes growing in the brackish- or fresh-water marsh associes.

The scrub mangrove facies. In unfavorable locations the Avicennia salt-marsh associes is reduced to a dwarfed stand of *Avicennia* with dwarfed *Laguncularia* and *Rhizophora.* The dwarfed trees are widely spaced, and the associated salt-marsh herbage and shrubs are sparse. This community occurs in two distinctly different situations: in paludal basins where the soil level is low and the swamp flooded by stagnant water; and on elevated soils above the usual tide level or along the margin of the high-tide level. Both these situations, and the community, are locally known as mangrove flats or salt-marsh flats. The former of these scrub mangrove facies of flooded soils seems to be the result of extreme conditions of salinity and poor aeration of the surface water. The water over the swamps is not regularly changed by the tides or currents and becomes very salty during some periods. At other times it is diluted by rain and becomes almost fresh. The soil-solution salinity varies less but is usually high. The soils themselves are most often peat or muck. There seems to be a slow subsidence of the soil level, probably due to decomposition and compacting of the organic soils. Although *Avicennia* is the dominant tree of the usually adjacent Avicennia salt-marsh community, it is much reduced in both number and size in this facies. The dying *Avicennia* trees in or along the margin indicate that it was probably an Avicennia salt-marsh associes whose codominants have increased with more intensive inundation. The community may be the result of a retrogressive change instead of a progressive, successional development. Large areas are covered by these flooded mangrove flats, and a better understanding of the conditions that produce them is needed. Table 2 gives most of the typical species.

The scrub mangrove facies of intermittently flooded soils (pl. 7) is very similar in growth form and essentially similar in dominants to the one of flooded soils, but salt-marsh plants are more frequent. The best examples of this type are on the thin soils over the coral and lime rocks of the Florida Keys. Most of the soils are heavy marl. The stand of dwarfed trees is very widely spaced and the salt-marsh herbage and shrubs cover most of the soil. Some of these alternately wet and dry soils are so salty that no vegetation covers them (see "Salinity"). The soils are not regularly flooded by every tide, and zonation around depressions that retain water is very striking.

Both these types of dwarfed mangrove growth are the result of conditions unfavorable to growth. Neither type contributes much to the soil accretions or seems to be in the direct line of the mangrove succession (see fig. 2).

Rhizophora locies of the brackish-water marsh associes. Along much of the southern coast the shallow flooded areas, known as glades, between the outer zones of red and black mangroves and the marl prairies, hammocks, or fresh-water marshes are covered by a very stunted growth of *Rhizophora* in a brackish-water marsh, associated with brackish-water species of plants. *Juncus roemerianus, Sporobolus virginicus,* and some species of *Spartina* are common herbs, and *Borrichia frutescens* is a common shrub. The red mangroves are few and widely spaced, and there is an even smaller number of *Laguncularia* bushes.

The water over these nearly flat areas is stagnant and materially altered by rainfall. As in the preceding scrub mangrove facies, the conditions for mangrove growth are unfavorable. The community contributes somewhat to the raising of the soil level, as some peat accumulates in it. It plays a relatively small rôle, however, in the mangrove succession.

Rhizophora locies of the fresh-water marsh associes. This community is not sharply demarcated from the former. As the marsh vegetation changes inland from a brackish-water to a fresh-water type, the red mangroves continue to be associated with it. The occurrence of *Rhizophora* in such situations is difficult to explain. The plants are dwarfed but do not appear to depend upon saline water or soils, as only the slightest traces of salt were found in either at many localities. The red mangroves are scattered over the marsh and contribute little, if anything, to the mangrove succession. The community may be considered part of a seral savanna. It is locally called prairie, marsh, or savanna, depending upon depth of water and number of small trees.

Zones of this type of vegetation, 4 or 5 miles wide in many places, occur along the southern coastal strip of the Everglades (pl. 4, fig. 2). Some residents of the region state that the mangroves in these marshes once extended still farther inland, and they consider drainage and fire responsible for their retreat toward the coast. In a number of places dead bushes were found farther inland than the present limits of this growth.

Sloughs, pools, and other depressions in the Everglades at some distance from the coast are frequently surrounded by colonies of *Rhizophora*. Such dominantly fresh or mildly brackish situations are often not directly connected with the mangrove swamp areas, and the occurrence of the trees is difficult to explain. They form local swamps near to or associated with almost any type of vegetation of the region. It is probable that salt water invades these areas during storms or unusually high tides, and that the mangroves require only this occasional salt to promote their growth.

The Associated Types of Vegetation

Most plant communities in Florida south of 29° north latitude are more or less intimately associated with mangrove swamps, because this part of the state is of such low relief that most of the vegetation is affected by coastal conditions. A number of these communities are part of a halosere similar to or associated with the mangrove halosere. Harper (1917, 1921) and Harshberger (1914) have described most of these communities, and McAllister (1938) has described Key Biscayne, Bowman (1918) the Tortugas Keys, and Millspaugh (1907) the Sand Keys.

The marine aquatics form a definite community in open, shallow water, which under favorable conditions is invaded by mangroves to form the pioneer community of the mangrove prisere. On the salt-free, non-flooded soils are a number of distinct types of vegetation all of which belong in the successional series, with the exception of the tropical climax. Between these two extremes are the mangrove swamps, the different types of marsh, and the seasonally wet prairies. In the fresh-water marshes and swamps are a number of distinct communities, a few of which include mangroves. The salt-water marshes are the communities most intimately associated with mangroves. The seasonally wet prairies and the strand communities also include some mangroves. Along the tension zones between distinct mangrove communities and these communities the flora is often very confused, and it is impossible in many instances to delimit the communities accurately. In some places the mangroves are definitely antecedent to some of the non-halophytic communities, but in other instances there is no apparent successional relation.

Of the non-flooded communities, four are most frequent and important. These are the tropical or subtropical climax association, known as hammock forests; the pineland edaphic subclimax associations, dominated by either of two species of pine; the south temperate climax association of hardwoods and palms; and the dune associes. Figure 2 indicates that the pineland and dune communities may develop into climax hammock forests, but this is problematical. Some of the hammock forests have developed from fresh-water or brackish-water marshes and swamps. A number of the hardwood and palm hammocks scattered over the Everglades are fringed by mangroves, and investigation of the soils of some of these showed that the hammock humus soils are in some instances underlain by mangrove peat, or by marl that seems to be of mangrove origin. Similarly, some of the hammocks of the fresh-water Everglades have developed from the marshes or fresh-water swamps, which are often a serclimax. The cypress is common in the glade marshes and seems to aid hammock formation.

The Hammocks

The subtropical and tropical forests are hammocks, composed of many species of broad-leaved trees and palms, of which a number of types have been described by Harper (1917) and Harshberger (1914). In southern Florida there is an abrupt change from a mixture of south temperate and subtropical flora to the tropical flora of some of the Florida Keys and parts of the mainland. The tropical hammocks seem to be confined to places where the temperature within them rarely or never falls below freezing. They are common southwest of Miami in the pinelands and in marsh areas. A few hammocks occur along the narrow Atlantic coast strip as far north as Brevard County. Many occur near the coast or on islands, where they are frequently closely associated with mangrove swamps. Most of the other types of hammock are inland. There are a few dune or shore hammocks which may be intermediate in position between the sandy foredunes and the interior hammocks, or between the dunes and the interior pinelands or marshes. Near the coast there are some interesting shell mounds, built by aborigines, that are covered by such hammock forests.

The tropical hammocks on the Florida Keys and along the coast have many typical tree and shrub species that are frequent associates in the Conocarpus transition associes, but in nearly all cases the plants are not well developed in the transition zone. A few of these are listed in tables 3 and 4. *Sideroxylon, Drypetes, Bursera,* and *Swietenia* are typical tree genera of the hammocks. A number of palms, as *Roystonea* and *Paurotis,* are locally abundant. There are many ferns, and the epiphytes are very characteristic. There are very few flowering herbs and grasses. Most of the tropical hammocks are on well-drained humus soils, but a few are on soils of low elevation, and in such hammocks the humus soils are in some places underlain by mangrove peat. This latter condition is particularly characteristic of some of the shore and Everglades hammocks in the region from the Dade County pinelands toward Cape Sable.

The Pinelands

The two types of pineland are very similar in appearance, being open flatwoods with a savanna-like stand of trees associated with grasses, shrubs, and scrub palms. The tropical pinelands are dominated by the Cuban or slash pine, *Pinus caribaea,* and the more temperate pinelands by the long-leaf pine, *Pinus palustris.* The former pine is commonest in southeastern Florida over the Miami oölite soils, and the latter covers large areas inland and near the coast, farther north, on sandy soils. The two species are found mixed in some of the intermediate pinelands. Both types of pine forest frequently grow on such low soils that they are adjacent to mangrove communities. However, in only a few places is the relation so close that either can be interpreted as affecting the other. There is usually a definite fresh-water marsh, dune, or other zone between mangroves and pineland, or else the pineland is on such elevated, well-drained soils that its distinctness from the swamps is very evident. The tropical pinelands of the lower Florida Keys, which are composed of limestone, are a possible exception, for they are an open stand on soils very similar to those of adjacent buttonwood and mangrove communities. The pineland soil here, however, is so thin that the mangroves can have had little or nothing to do with its accumulation.

The Strand Vegetation

The association of mangroves with strand plants is intimate, but the mangroves are not typically a part of the strand, which includes the wave-built beaches and dunes. A few mangroves occur as pioneers in the offshore shallows and may be effective in promoting beach and dune formation by lessening wave wash, but they become only temporarily lodged on the seaward faces of beaches and dunes themselves. In some places *Conocarpus* is common on the dunes above the usual storm-tide levels, and it has a definite place in the strand flora.

Mangroves grow on the lee side of islands or bars, or in swales between the dunes, and are effective in creating conditions favorable for the accumulation of sand and muck. Many of the islands along the coast, especially the Florida Keys, have colonies of *Rhizophora,* with a few of the other mangroves, on the flooded lee side where currents are not strong and finer sediments settle out of the quieter water. These are likely to become so thickly grown up that subsequent storms may wash in coarser materials and pile up beach and dune sands among the trees. This material may cover the mangrove roots to such a height that it kills the plants, and eventually soils accumulate well above normal high tide. Thus, in favorable places, all or part of a mangrove swamp may become converted into coarse sandy beaches and dunes. This participation of mangroves in the strand succession occurs best in depressions, or in nearly enclosed basins between bars or shoals that later form beaches, or in the flooded swales between dunes. Soils accumulate in such places and the enclosures become filled with sand and organic materials. This seems to be the mode of origin of the ponds (Bowman, 1918) on Bush Key of the Dry Tortugas, and is the cause of a number of mangrove and buttonwood colonies within the broader expanse of the strand vegetation of the Florida Keys and Ten Thousand Islands.

Beaches and dunes are more common along the Florida coasts as a whole than are mangrove swamps. The conditions which produce sandy beaches are not common in the mangrove areas of southern and southwestern Florida, and the two types of vegetation are not well developed along the same coastal regions. Where there is strand vegetation in mangrove regions, it is on low, narrow shell or sand ridges. Some of these are between the flooded parts of the swamps and the interior partly flooded salt marshes with black mangroves and buttonwoods.

Thus a transect from open water inland may include a strand associes between the mature Rhizophora consocies and the Avicennia salt-marsh associes.

The strand flora is also halophytic and includes some of the plants common to mangrove swamps, as *Sesuvium portulacastrum* and *Sporobolus virginicus,* but its typical species, such as *Ipomoea pes-caprae, Uniola paniculata, Suriana maritima,* and *Tournefortia gnaphalodes,* are not normally able to withstand constant flooding by salt water and are not frequent in swamps. The list of strand plants on page 344 includes only about a third of those of frequent occurrence, and does not include some that may be abundant in some localities. There has been some tendency to list the mangroves with the strand plants, but it seems better to exclude them from this classification.

The Fresh-Water Marshes and Swamps

Thousands of square miles near the coast are covered by salt-water, brackish-water, and fresh-water marshes. Temperate-climate salt-water marshes extend southward along both Florida coasts, decreasing in abundance southward as the mangroves become more prevalent. Fresh-water marshes cover even larger areas of the peninsula, as so many of the central as well as coastal parts are of low relief. The Everglades is the largest of these marsh areas (pl. 8), and contacts mangrove swamps along many miles of its border. Brackish-water marshes are intermediate between saline and fresh-water marshes and contain floral elements of both.

Fresh-water swamps are common inland, but few are near enough to the coast to merge with mangrove swamps. A few occur along the low coast of the Gulf hammock region from the Onclote Keys to the Cedar Keys. Those near Crystal Bay were studied. They are great river swamps that extend out to the brackish-water marshes, but so few mangroves occur in the adjacent marshes that there is no actual zone of contact.

Cypress swamps of scrubby trees form a large forest area in Collier, Hendy, and Lee counties on the western edge of the Everglades. Smaller cypress areas exist along the southern edge of the Everglades. These are composed mainly of the stunted form of *Taxodium ascendens,* and typically form island-like, dome-shaped communities in the broad expanse of the marsh. Many of these clumps seem to be incipient hardwood hammocks; Curtis (1888) considered the cypress and mangroves the important agents that build up the soils of the peninsula. There are only a few areas where these cypress swamps occur near mangroves. Some of the southern coastal marshes have small but well-defined colonies of cypress with red mangroves bordering the colonies and scattered over the fresh- or brackish-water intervening marsh. The mangroves may help to build up the soils suitable for the cypress, and these in turn may build it up to the hardwood hammock stage. No detailed study was made of this possible succession.

Penfound and Hathoway (1938) in their study of the marshlands of southeastern Louisiana recognized the gradual changes in flora from a strictly fresh-water to a definitely salt-water marsh, and they found wide ranges of tolerance for salt in many species. Similar conditions are found in Florida. The most constant and frequent species of the salt-water and fresh-water marshes, given on page 344, may, with few exceptions, be found in brackish-water marshes.

The Salt-Water Marshes

The salt-water marshes are most intimately associated with mangrove vegetation: the northern extent of the mangroves is as scattered individuals in salt marshes, and the southern extension of salt marshes is as small colonies, or an under story of vegetation, in the mangrove swamps. Both have nearly identical requirements of water, soil, and tide conditions, and afford a good idea of the intimate alternation of two types of plant community. Low temperature is the critical factor that limits the northern extension of the mangroves; on the other hand, most of the salt-marsh plants range from the tropical to the north temperate zone, and few are definitely temperate-zone species. In many cases it seems that the salt-marsh vegetation decreases southward because of increasing competition with mangroves as well as because of temperature differences. The halophytic species of *Spartina* and *Juncus* do not survive in thick swamps. Of the many salt-marsh plants only a few, *Batis, Salicornia, Dondia,* and *Borrichia,* are of constant enough occurrence to be significant. A short list of the important salt-marsh species is given on page 344.

The red mangrove can begin growth where there are no emergent marsh plants, and once well established can withstand deeper flood waters than marsh plants. Therefore, mangroves cover a wider zone of the littoral than do the salt- and brackish-water marshes. The grassy salt-marsh plants, particularly *Spartina alterniflora, Spartina cynosuroides, Juncus roemerianus, Sporobolus virginicus,* and *Monanthochloë littoralis,* form large, nearly pure consocies in open marshes or occur as enclosed socies within mangrove swamps. Of these species, *Spartina alterniflora* grows best in the outer deep-water zone of swamps and may be effective in holding soil materials, thus aiding in establishment of a pioneer Rhizophora family. Such a pioneer growth of grasses is more commonly the case in sandy-soil regions than elsewhere.

The succulent marsh shrub halophytes, as *Salicornia* and *Batis,* are found in the most salty situations that support plant life. The salt-marsh leather fern, *Acrostichum,* is common in all types of marsh and swamp, and even in the hammocks. The débris from many marsh plants, especially in the Avicennia salt-marsh associes, is considerable and probably contributes materially to the accumulations of peat.

On the west coast from Tampa Bay southward, and particularly on the lower Florida Keys, there are many "salt flats," flat expanses of firm sand, or thin marl over limestock rock, close enough to sea level to be inundated at high tides, which support a sparse covering of salt-marsh plants and occasional mangrove bushes. Such vegetation may be considered either a salt marsh or a scrub mangrove facies, depending upon the relative abundance of marsh and mangrove plants.

Throughout south Florida there is no marked boundary between salt marshes and a mixed growth of mangroves and salt-marsh plants. The two communities are also similarly related to fresh-water marshes. Even a seasonally wet prairie type of vegetation is frequently found near them. A rigid classification of salt-marsh vegetation into distinct communities is unwarranted at many places. The dominance of mangroves in or near salt marshes seems in many instances an accident of distribution of mangrove seedlings, for with nearly identical habitats there are great differences in the relative abundance of mangroves and marsh plants at different localities.

Representative Species of the Strand, Salt-Water Marshes, and Fresh-Water Marshes

(Twenty species of each selected without regard to their abundance in any area but according to their frequency. Woody plants not listed for the fresh-water marshes.)

Strand Vegetation— Beach and Young Dunes	*Salt-Water Marshes*	*Fresh-Water Marshes*
Cakile fusiformis	Acrostichum aureum	Acnida australis
Cenchrus tribuloides	Batis maritima	Blechnum serrulatum
Chamaesyce buxifolia	Borrichia frutescens	Chaetochloa magna
Coccolobis uvifera	Baccharis halimifolia	Crinum americanum
Conocarpus erecta	Distichlis spicata	Dichromena colorata
Croton punctatus	Dondia linearis	Eleocharis cellulosa
Distichlis spicata	Fimbristylis castanea	Eupatorium capillifolium
Helianthus debilis	Iva frutescens	Gyrotheca tinctoria
Ipomoea pes-caprae	Juncus roemerianus	Hydrocotyle umbellata
Iva imbricata	Kosteletzkya virginica	Mariscus jamaicensis
Jacquinia keyensis	Lycium carolinianum	Nymphaea macrophylla
Lantana ovatifolia	Monanthochloë littoralis	Oxypolis filiformis
Scaevola plumieri	Panicum virgatum	Panicum hemitomon
Serenoa serrulata	Rhabdadenia biflora	Phragmites communis
Sesuvium portulacastrum	Salicornia perennis	Pluchea foetida
Sophora tomentosa	Spartina alterniflora	Pontederia cordata
Suriana maritima	Spartina cynosuroides	Rhynchospora corniculata
Tournefortia gnaphalodes	Spartina patens	Scirpus californicus
Uniola paniculata	Sporobolus virginicus	Spartina bakeri
Yucca aloifolia	Typha angustifolia	Typha latifolia

The Prairies

Open glades of herbs and low shrubs with a few scattered trees or island-like hammocks are common over many of the more elevated portions of the Everglades and over part of the southern coastal region. Where the soils are seldom flooded, this vegetation is locally known as a prairie. In many instances such prairies have the aspect of a savanna. Such a prairie may not differ greatly from a flooded marsh, and may seasonally be as wet as a marsh.

In general there are two types of prairie, the seasonally wet prairies of depressions in or near the pinelands, and the marl prairies. The former are seldom near mangroves, and the latter are closely associated with mangroves, especially the Rhizophora locies of the brackish-marsh associes. The southern coastal prairie described by Harper (1917) is an extensive strip from near the shores of Biscayne Bay to Cape Sable, and forms a modified, marl-soil part of the Everglades; the southern part of the Everglades has a limestone or marl substratum instead of sand. The saw grass, *Mariscus,* is present but is usually sparse and dwarfed. There are many clumps of small trees, possible incipient hammocks, and small depressions or creeks with marsh vegetation or mangroves. A few scattered red mangroves occur over the more saline, most flooded portions of the prairie. In the Cape Sable region buttonwoods, palms, and low grasses are common. So many acres of this coastal prairie have been drained and cultivated that over large areas the vegetation is a weed growth of many introduced species, of which the large *Acnida* is typical.

The Southern Coast Complex of Vegetation

The whole complex of vegetation along the southern coast of Florida is very difficult to interpret. Nearly all the above types of vegetation occur in close proximity. A number of types of tropical hammock, and clumps of hardwoods and palms that may be incipient hammocks, dot the broader expanses of the marshes and marl prairies. The Miami oölite pinelands are dissected by transverse glades of marsh or prairie vegetation. From the edge of these pinelands to the coast, especially between Royal Palm Hammock (Dade County) and Cape Sable, is a maze of watercourses, ponds, and lakes of brackish to fresh water, bordered or covered by mangroves. In the shallow flooded marshes occur a variety of plant communities. In some places *Taxodium* is the dominant tree, in other places there are scattered dwarfed red mangroves. *Typha* predominates in some places and *Mariscus,* the saw grass, in others. Krome Prairie (north of Cuthbert Lake) and the land west of Whitewater Bay, and much of the territory along the state highway from the pinelands southwest of Homestead to Cape Sable, are typical of this complex of vegetation. A few inches difference in elevation and a slight difference in salinity of the surface water or soil seem to be the causes of the variation. Some of the best zonation of the mangrove swamps is found in this region, although there is little or no tide. Mangroves are mixed with many other communities over this wide belt along the southern coast, and their presence is difficult to explain.

The Submarine Vegetation and Marine Animals

Marine algae, some genera of the lower orders of seed plants, and many kinds of invertebrate abound in the shallow water along the southern and Gulf coasts. Coral reefs occur along the outer, clear-water part of the chain of the Florida Keys, and they form the Florida Reef Tract. These reefs are too far offshore and in water too deep to be associated with mangroves, but débris from them washes in to the shore and is of some importance in forming soil materials in the swamps or on the shoals on which mangroves pioneer. Long Key of the Tortugas Keys is a coral-ballast shoal on which mangroves have begun and on which many have been planted.

The most significant submarine community associated with mangroves is the marine aquatic associes of plants, with some typical animals. This vegetation covers large areas in Florida Bay, Barnes Sound, and most of the other shoal waters of that region. At low tide some of the plants are exposed, and they extend from such shallow water to depths of 20 to 30 feet. The turtle grass, *Thalassia testudinum,* and in some places the manatee grass, *Cymodocea manatorum,* form large banks of vegetation over marl or shell sand bottoms in shallow water. With these are associated a few other seed plants, *Halodule wrightii, Halophila,* and *Ruppia.* Many genera of marine algae, *Sargassum, Halimeda, Caulerpa, Udotea, Penicillus, Gracilaria, Polysiphonia, Acetabulum,* and *Batophora,* are represented. Sponges, many mollusks, some small crustaceans, and some brittle stars are common.

Drifting mangrove seeds and seedlings, particularly the long seedlings of *Rhizophora,* become entangled with these plants, and if held in place long enough to send down roots into the soil, become anchored to begin a mangrove community. Furthermore, the leaves, rhizomes, and thalli of these plants break away or are rooted out by fish and float about in large masses; some species of floating algae, *Sargassum natans* and *Sargassum fluitans,* add to this surface drift; and this material washes into the swamps or upon the shores in large quantities, adding an important amount of organic débris to the soils. Much of the humus of sandy shores consists of the remains of these plants. Mangrove seeds and seedlings become entangled with the floating mass and may be carried greater distances than they would travel alone. In some instances it becomes so thick that they take root in it while it is still floating.

Miner (1937) and many marine biologists have noted the great number of animals associated with the plants of this submarine community and the mangroves. The sections on biotic factors of the environment and biotic factors in soil accretion give more details of this abundant marine littoral animal life.

ENVIRONMENTAL FACTORS

Introduction

A significant feature of mangrove swamps is the very complex nature of their partly marine and partly land habitat. Many factors of the environment operate so closely together that it is difficult to distinguish the effects of any one from those of others. This is particularly true of the marine factors, in which there is almost constant change owing to the tides. A considerable body of data was collected about some of the major factors, and these data can be correlated with those concerning many features of the vegetation and soil accretions. There was insufficient time and inadequate equipment for the measurement of some of the less obvious factors. Moreover, an ideal ecological study should include a detailed investigation of the requirements of the dominant species; in other words, it demands a series of autecological studies as well as a study of environment. Since the dominant species of mangrove swamps are more distinct than those of most plant communities, a study of the environmental factors of a mangrove community is in many instances a study of conditions of growth of the dominant species. Bowman (1917) made significant studies of some of the requirements of *Rhizophora mangle,* which reflect the requirements of some parts of the swamps. There is, however, not much literature on the environmental factors of mangrove swamps, as most of the writers have concerned themselves with the vegetation and made only general observations on such factors as salinity, tides, and soils.

Salinity of the water and soil solution; the tide; the water level, whether caused by tides, rainfall, or drainage from the interior; and the soils are the major environmental factors. The climate, drainage, aeration, and the associated biota are somewhat less important. In addition to these habitat factors, the factors that influence soil accretion are of particular importance in this study. These include certain marine currents and physicochemical conditions that affect the vegetation very little. They are considered in part II of this paper. However, several of the marine factors are important both in the environment of the swamps and in the sedimentation processes, and must be considered both in this and in the latter part.

SALINITY

Both surface-water and soil-water salinity are very important. In mangrove swamps there is a range from water with a salinity much greater than that of sea water to water that is so nearly fresh that the small trace of sodium chloride can be detected only by titration. Mangroves are halophytes, in the general sense of that term, growing most abundantly where the concentration of sodium chloride is more than a third that in sea water. But they are not absolutely conditioned to any narrow range of salinity. The experimental cultures in this investigation, and the work of Bowman (1917), show that seedlings of *Rhizophora, Avicennia,* and *Laguncularia* can be grown for over two years in salt-free water, and in some cases in salt-free soil and water. Some of the experimental plants show no ill effects of a non-halophytic environment after 3 years' growth, and continuance of the cultures may show that the plants can grow to maturity without salt. If so, mangroves are facultative to water and soil conditions. In the natural environment, this condition of little or no salt occurs in the savanna-like marshes where scattered *Rhizophora* bushes grow.

There has been some speculation on the restriction of mangroves to the saline marine littoral zone. Watson (1928) advances the theory that mangroves with their vivipary are old residents of the seacoast, having persisted in this narrow zone because of their inability to penetrate farther inland. However, some relatives of the mangroves grow inland beyond the reach of salt water, and, in the absence of much paleontological evidence, it cannot be stated positively that mangroves have inhabited the coast for a long time. Watson's assumption seems based too much on the one fact of vivipary. Some students are not inclined to consider vivipary a primitive character, and certainly mangroves belong to higher orders of plants than those usually considered primitive. It seems more likely that mangroves are descendants of land forms that have invaded the sea, and, as J. H. Davis (1938*a*) states: "What happened to force these back into salt water among the primitive algae and animals can only be surmised. If it was ruthless competition they certainly now grow where no other plants dispute their domain." Their apparent preference for saline conditions seems to be largely a way of escaping competition, but whatever the reason for their peculiar distribution, they have the typical xeromorphic adjustments to salt conditions.

Methods of Study

Rough calculations of salinity of the water were made by adjusting specific-gravity readings to percentage of salt, using standard hydrometers, thermometers, and conversion tables. In addition, the more exact method of titrating the water or soil solution was used. The chloride was determined by titrating with silver nitrate and the sodium chloride content calculated. In many instances the same sample of water was tested by both methods, and the results were in nearly all cases sufficiently comparable for the data from either method to be considered reliable. Only the titration method could be used to determine the soil salinity (Chapman, 1939). Summaries of water salinity are given in table 5, and of soil-solution salinity in table 6.

TABLE 5. *Average salinity of the sea, shallow waters, and surface waters in the mangrove swamp communities*

(Percentages calculated from hydrometer readings and titration with silver nitrate)

LOCATION OR COMMUNITY	NO. SAMPLES	SALINITY (PER CENT)			
		1936	1937	1938	Average
OPEN SEA NEAR SHORE					
Atlantic Ocean	5	3.70	3.68	3.71	3.70
Straits of Florida	16	3.57	3.62	3.78	3.62
Tortugas	12	3.58	3.57	3.61	3.59
Gulf of Mexico	10	3.17	3.31	3.25	3.26
BAYS, SOUNDS, SHALLOWS					
Biscayne Bay	9	2.19	3.52	3.78	3.08
Barnes Sound	15	2.54	3.87	3.66	3.17
Jewfish Creek	12	2.49	3.42	4.28	3.42
Surprise Lake	15	2.53	3.74	4.36	3.53
Florida Bay	22	2.67	3.55	3.92	3.37
10,000 Islands	9	1.72	....	2.88	2.19
Lemon Bay	6	....	3.31	3.37	3.34
Sarasota Bay	6	2.85	....	3.85	3.28

(*Continued on following page*)

TABLE 5—*Continued*

LOCATION OR COMMUNITY	NO. SAMPLES	SALINITY (PER CENT)			
		1936	1937	1938	Average
ESTUARIES					
Banana River	4	1.80	....	....	1.80
Indian River	10	1.31	2.85	....	1.58
Shark River	6	....	2.69	2.75	2.70
Broad River	4	0.97	....	....	0.97
Chatham River	8	0.94	....	1.04	0.96
Turner River	6	....	....	0.89	0.89
Baron River	5	0.38	....	1.38	0.88
Manatee River	6	1.40	2.35	3.12	2.27
RHIZOPHORA COMMUNITIES					
Pioneer Rhizophora family	37	2.123	3.012	3.712	3.135
Mature Rhizophora consocies	47	3.139	3.881	3.445	3.488
Rhizophora brackish-water marsh associes	11	1.371	1.991	1.317	1.556
Rhizophora fresh-water marsh associes	10	0.001	0.007	0.009	0.008
AVICENNIA SALT-MARSH COMMUNITIES					
Dominant Avicennia	28	2.107	4.101	3.865	3.684
Dominant salt marsh	22	3.176	3.896	4.217	3.863
MIXED MANGROVE AND LAGUNCULARIA COMMUNITIES					
Mature mangrove forest associes	27	1.070	1.564	1.136	1.153
Scrub mangrove facies	31	3.225	4.437	5.074	4.576
Laguncularia consocies	9	0.975	1.818	1.376	1.289

TABLE 6. *Average salinity of soil solutions compared with some surface-water salinities in mangrove and associated communities*

(Percentages calculated from titration with silver nitrate; estimation of the soil solution by oven-drying soils)

COMMUNITY	NO. SAMPLES	SALINITY (PER CENT)	
		Soil solution	Surface water
Pioneer Rhizophora family	7	2.91	3.185
Mature Rhizophora consocies	14	3.56	3.488
Avicennia salt-marsh associes	13	5.11	3.684
Mature mangrove forest associes	5	2.21	1.153
Scrub mangrove facies	15	8.42	4.576
Conocarpus transition associes	8	1.80	0.144
Dune strand associes	17	0.94	Normally no surface water
Tropical forest climax association (only those adjacent to mangroves)	8	0.35	Normally no surface water

Most of the water samples were taken and tested with the hydrometers while in the field. Subsurface as well as surface waters were obtained. Water samples for titration to determine the chloride, carbonate, bicarbonate, and total solids were taken to the laboratories. The methods in all cases were as standard as it was possible to make them. The *Standard methods of water analysis,* 1933 edition, was used for water. The soil samples were weighed, dried, and weighed, and their solutions titrated. The amount of chloride salt was then calculated for the total soil and for the soil solution. How much of this soil solution is available to or influences the plants as free soil water is not known. The figures for soils are, however, significant, although they are less numerous and less significant than those for water. With records from all the main mangrove communities, the open sea, shallow basins, and rivers, the data are adequately comprehensive. Fortunately the seasons of 1936 and 1938 were unusually wet and dry respectively and the season of 1937 was about normal for rainfall, so that the effects of seasonal differences in rainfall could be observed.

Results and Discussion

The first and most obvious condition is the slight range of salinity in the open sea, and the great range in the shallow bays and sounds (table 5). Few or no seasonal changes occur along shores of the deep waters of the Atlantic, Gulf, or Straits of Florida because of currents, winds, and the normal tide exchange that is effective far into the interior of the swamp. The data from open water show that there is a gradual decrease in salinity from the Atlantic waters (3.7 per cent) through the Straits of Florida by way of the Tortugas Keys and into the Gulf of Mexico. Along the shores of the Gulf the water is often barely 3.2 per cent saline, and the Tortugas water is approximately 3.6 per cent saline.

In contrast, some of the nearly enclosed bays and sounds, as Card, Barnes, and Blackwater sounds, and Florida, Chokoloskee, Sarasota, and Tampa bays, receive so much fresh water from the mainland during the rainy season that they may become very dilute, and at another season, owing to intense evaporation, they may become more concentrated. In Barnes Sound and Florida Bay salinity was over 1 per cent greater in 1938, a dry year, than in 1936, a wet year. In some shallows the water was over 4 per cent saline in 1938. Although there is this great fluctuation in salinity, it is around the shores of such bodies of water that the best mangrove communities are found.

The pioneer Rhizophora family and the mature Rhizophora consocies occurring along the outer parts of low, gently shelving shores and on islands and shoals in the bays have a fairly uniform salinity. Even though in exposed situations in shallows, the pioneer communities of relatively open water have a wider range of salinity than the mature Rhizophora consocies because of more intense evaporation and dilution of the shallow, exposed water. Some of the Rhizophora communities back from the coast are flooded by almost fresh or mildly brackish water. The brackish marshes with scattered dwarf red mangroves average just 1.55 per cent salinity, and the unique communities of scattered dwarf red mangroves over parts of the southern Everglades grow in water with only the slightest trace of salt, and sometimes with none that could be detected.

The Avicennia communities tolerate the highest concentrations of salt in the surface waters and in the soils. Scrub mangrove communities of *Avicennia* and salt-marsh plants frequently occur on partly flooded flats where the soil water may be over 8 per cent saline and the shallow surface waters over 5 per cent. These communities are seldom as deeply flooded as the mature Rhizophora consocies and may go for long periods without being flooded by the normal tides. The shallow waters evaporate quickly and the salts are concentrated. The soils may retain enough of this salt to keep them saltier than the surface waters. In fact, hard rains dilute the surface waters so much that they have a wider range of salinity than in any other community.

Avicennia communities typically contain marsh halophytes that tolerate saltier situations than does *Avicennia*. Over some of the thin marl soils of the Grassy Keys, Saddle Bunch, and other keys near Key West the dwarfed black mangroves occur around depressions that are alternately filled with tide or rain water and evaporated to dryness (pl. 7). The mangroves do not, however, extend over the saltiest of these places, as do *Salicornia* and *Batis*. In some places the saltiest soils, which frequently have 10 per cent salinity, remain bare of any vegetation. The apparent zonation from a mature Avicennia salt-marsh associes, with a few marsh plants, through scrub Avicennia flats with more marsh plants and stunted black mangroves, to an open salt marsh, and in some cases finally to bare areas, seems correlated with increase in the soil salinity if not in the surface-water salinity. In many instances there is no standing water over these zones and only occasional flood waters from high tides or rains.

Laguncularia has a wide tolerance for salt, occurring in scrub flats that may become very salty, or as consocies where the surface water and soils contain less salt than is typical of either Rhizophora or Avicennia communities. In the Memphis laboratory, cultures of *Laguncularia* plants grown in fresh water have definitely less xeromorphic leaves than those grown in sea water. However, nearly all these plants have died after 18 months under salt-free conditions.

The Conocarpus transition associes is not normally flooded by the tides and in many localities has no surface water. Where there was water, the tests showed an average of less than 0.2 per cent salinity. In some places and for a season, however, the soils of this transition zone may be very salty. This is especially the case where *Conocarpus* forms a zone between the bare salt flats, or salt marshes, and the slightly elevated upland vegetation.

From the above, and the data in tables 5 and 6, it is evident that each of the plant communities of the mangrove succession has a more or less typical average salinity and range of salinity. The Avicennia communities are the most saline and tolerate a wide range of salinity. The Rhizophora communities of normal-sized plants are typically of the same salinity as the open waters adjacent to or around them. Since these swamps are well flooded by the tides, they have a narrow range of salinity. However, dwarfed *Rhizophora* can exist in brackish-

and even fresh-water marshes of little or no salinity (pl. 4, fig. 2). *Conocarpus* grows where there is usually very little salt. *Laguncularia* has a wide range of salt tolerance.

In the mature mangrove forest associes along the southwest Everglades coast the surface water is mildly brackish and may be nearly fresh during the spring and summer rainy season. The average summer-season salinity of these swamp waters is about 1.15 per cent, which is one-third the salt concentration of sea water. In general the best growth of any of the mangroves seems to be where the water salinity is between 1 and 2 per cent. The maximum tolerance for salt is probably the limiting factor in the distribution of these plants, and the average or minimum salinity is of less significance. The highest concentrations of salt are in the soils. The lower levels of water in the estuaries and shallow bays are saltier than the upper levels, but this affects only the few mangroves lining the shores, whose roots can obtain the higher concentrations of salt from these lower levels. Harshberger (1914) considered the upstream movement of salt water along the bottom of the estuaries important. It may account for the narrow fringe of mangroves some distance inland along the rivers, but it is of little significance in the large swamps flooded by only a foot or so of the upper layer of water from the rivers or the sea.

The adjacent types of vegetation are not all typically adjusted to salt conditions, but all of them seem to be able to withstand some fairly regular to exceptional occurrence of salt in the water or soils. This is particularly true of the strand communities. The upper beach and dune communities have soils seldom if ever free of salt. Many analyses of the strand soils of the Tortugas Keys show that not only do high waves wash salt water over the upland, but it is probable that the salt spray during high winds brings about the gradual accumulation of salt in the soil even where waves never cover it. Some salt in the soil many miles inland is common over much of south Florida where it has not been leached out. Hurricanes no doubt are capable of blowing salt spray far inland and thus spread salt well beyond the inner margins of the highest tides and storm waves. A few soil samples from the interior of hammock forests and pinelands 5 and 6 miles inland had traces of chloride, so that it is probable that none of the communities adjacent to mangrove swamps are continually free from the influence of salt. Occasional high water and storms may thus limit the seaward extension of these non-halophytic communities and materially check their encroachment upon the mangrove communities.

Harris (1934) and co-workers obtained very significant data on the osmotic concentration of plant saps expressed in atmosphere pressure. As his studies included plants of mangrove and associated communities in Florida and Jamaica, some of his figures on the osmotic concentration of cell saps are borrowed for comparison with soil-solution and surface-water salinities determined in this investigation. Although the number of mangroves and associated plants tested by Harris is too small to be as representative as the salinity data, the comparisons given in table 7 show some correlations that seem significant in this ecological study. In the interpretation of halophytes the correlations between the plant saps and habitat salinities may be most instructive (Chapman, 1936).

With the above in mind, and some similar studies of salt-marsh areas such as the investigation of the marshes of southeastern Louisiana by Penfound and Hathoway (1938), some general conclusions regarding salinity may be stated:

1. Salinity fluctuates widely with the seasonal rainfall, and year-round studies are needed to determine the range of conditions in Florida.

2. Only a few salt-marsh and mangrove plants are halophytes that definitely depend upon high salinity. Most of the mangroves are facultative to a wide range of salinity. A brackish condition is most favorable for the optimum growth of mangroves.

TABLE 7. *Comparison of osmotic concentration of saps in atmospheres (data from Harris) with per cent salinity of surface water and soil solution in mangrove and associated plant communities*

COMMUNITY	AVERAGE P (ATM.)*	SALINITY (PER CENT)	
		Surface water	Soil solution
Mature Rhizophora consocies	25.4	3.488	2.91
Avicennia salt-marsh associes	39.2	3.684	5.11
Conocarpus transition associes	18.7	0.144	1.80
Dune strand associes	19.7	....	0.94
Tropical forest climax association	18.5	....	0.35

*Figures in this column from Harris (1934).

3. Although the different species of the mangrove swamps and salt marshes have a wide range of tolerance, the average salinity of the communities is fairly definite, so that zonation corresponds to seasonal averages of the soil-solution and surface-water salinity.

4. The soil solution usually is more saline and fluctuates less than the surface water.

5. The highest salinity is found where the water level is close to the surface of the soil, with consequent high rate of evaporation. Some of the salt-marsh plants withstand higher salinities than any of the mangroves.

6. Saline conditions of both surface water and soil water extend farther inland than the normal range of the tide. The low relief of the land prevents rapid leaching out of this salt.

SOILS AND SUBSTRATA

There are three primary or basic soils on which mangroves begin growth, namely, (1) siliceous sands, (2) calcareous sands, (3) calcareous marl muds. These are sedimentary in origin and cover most of the uplands of Florida as well as the littoral zone and part of the sea floor. Cumulose organic soils of peat and muck accumulate on top of or mixed with these three primary soils wherever conditions of flooding along the coasts or in the interior promote marsh and swamp growth. Most of the underlying rock of the state is overlain by a few to many feet of siliceous sand. Near the coast this sand is mixed with calcareous materials, shell fragments, and different marine muds. In some sections the underlying rock is limestone, and where near the surface affects the surface soils. This is particularly true in the great Miami oölite limestone region of southeastern Florida and on some of the lower Florida Keys from Bahia Honda to Key West. There is some limestone near the surface along the middle west coast, but it does not materially affect the coastal soils.

North of the level of Lake Okeechobee siliceous sands predominate near the coast, but south of that point marl muds and other fine sediments increase in abundance. Mangroves occur on these wet coastal soils and on porous rock of limestone or coral, as well as on shoals formed of shell or coral fragments. Thus mangroves grow on substrata and soils varying in texture and structure from nearly solid rock to very fine-particled muds.

Since there are few detailed soil surveys of the Florida counties that have most mangrove growth, little is known of the mangrove soils. Harper (1921) briefly describes some of the soil conditions of southern Florida. Hammer (1929) and Waksman and Stevens (1929) give some interesting analyses of the Everglades soils, which show definite correlations between the dominant vegetation and the type of organic soil. Slow decomposition processes are indicated by the chemical analyses showing the fractions of organic complexes. Waksman and Stevens describe these Everglades peat soils as an example of lowmoor peats. They are similar to the organic soils of mangrove swamps and in some cases are mixed with mangrove peats.

The mangrove peats and mucks belong to a maritime group of soils composed of marine humus and sediments from the sea bottom and the cumulose materials of the swamps. To these soils the name "halotropic" has been applied. Stokes and Roberts (1934), describing similar soils in Puerto Rico, define the term as one "to designate the fact that profile development is related to salt water and brackish water by the effective activity of plant communities associated in a successional trend." The soil-profile and vegetational studies of this investigation clearly indicate that there is such a successional trend.

The diversity of soils or hard substrata on which mangroves grow is striking. This is due to their ability to grow where there is little actual soil, for their roots are bathed in water containing the essential nutrients, so that the soils and substrata are mainly for anchorage. There are few rocks that do not have many eroded pockets and crevices for the anchorage of mangroves, but shifting sands do not allow permanent anchorage. The nature of the soils is not effective in determining the kind of mangrove community, as all the common communities are found on every one of the types of soil.

A modified Hiler peat borer (pl. 5, fig. 1), soil augers, and a tubular-type sampler with flaps were employed to obtain soils from different levels and in different zones of the swamps. The Hiler slit-side sampler was effective in fibrous peat soils, but no good instrument was available for the plastic materials. In a few places where the soils were above the water table, a spade was used and the profiles were exposed without much alteration. In some cases the samplers were driven 15 feet into loose soil that was flooded by 2 or 3 feet of water, and it was difficult to obtain unpolluted or undisturbed samples under such conditions. Nevertheless there is enough obvious difference for good comparisons between the layers of the soils and between the kinds at different places. In most places visual inspection of the soils showed their character without chemical and physical analyses, but analyses were made on over 100 soil samples to supplement the field inspection. These chemical analyses (table 8) did not prove particularly significant and were discontinued after the season of 1936.

The general physical and morphological features of the soils are very different in different swamps. Thus, swamps with cumulose soils have distinct deposits of peat and muck, whereas in sandy and marl soil the mangrove roots may penetrate deep layers of homogeneous sediments without any distinct soil layers and no organic remains that indicate the type of vegetation. These characteristics are helpful in determining how the soil accretions occurred and will be discussed later (see "The Soil Profiles and Their Significance").

The pioneer Rhizophora family and the mature Rhizophora consocies occur on both marl and sand soils and on the loosest and coarsest shell-fragment shoals. Some of the mature red mangrove swamps are on deep peat soils, and others are on nearly bare rock with only small pockets by which the roots can anchor the plants. Heavy marl soils are commonest in the Avicennia salt-marsh associes, particularly the scrub mangrove facies. Both the coarse calcareous and finer siliceous sands are basic soils for all the mangrove communities. Peat and muck soils occur with many of these basic soils. If the materials are more fibrous than plastic the soil is a fibrous peat, and if more plastic than fibrous it is a plastic peat. If the organic matter is definitely less than half the soil material and is plastic, the soils are mucks.

Interbedding of soils is common in marl areas, where the fine plastic marl washes over and settles out on top of the other soils. In sandy-soil regions there is little interbedding, as the sands are not easily washed over the swamps.

The chemical analyses of these soils include loss on ignition, total carbon, calcium carbonate, and total nitrogen, but unfortunately two sets of analyses were made and the same procedures were not used in both instances, so that the data are not similar. The analyses of one set of samples do not show loss on ignition and organic carbon. Although these differences may be confusing, the results of some of the analyses are given in table 8 with the data grouped under three types of soil and the stations assigned to the regions indicated in figure 1. The loss on ignition and organic carbon both indicate organic content, but the loss on ignition is frequently greater than the percentage of plant materials, as some of the carbon of the calcium carbonate is included with it in some samples. The calcium carbonate content of some samples is very high, even in nearly pure

TABLE 8. *Chemical composition of different types of soil at selected stations*
(Percentages)

Stations	Loss on ignition	Organic carbon	Calcium carbonate	Total nitrogen
FIBROUS MANGROVE PEAT				
Regions A and D:				
MI 1	61.04	5.10	16.16	0.71
MI 2	69.14	8.94	16.84	1.18
MI 4	59.45	4.89	11.16	0.52
SB 2	....	....	1.79	0.69
SB 5	....	....	0.28	0.25
Regions B and C:				
KL 1	54.27	3.25	4.33	0.45
KL 2	72.83	10.83	5.27	1.26
KL 3	68.75	7.36	7.21	0.97
CC 1	....	....	6.26	1.31
CC 3	....	....	23.13	1.41
BR 3	....	....	1.33	7.65
TI 4	71.31	9.65	21.39	0.84
Average	65.20	7.14	7.10	1.38
MARL				
Regions B and C:				
KL 3	34.75	2.35	82.6	0.544
KL 5	25.24	0.18	32.04	0.441
FB 6	32.38	0.24	47.1	0.319
FB 12	28.40	2.74	62.5	0.875
CS 8	....	....	94.6	0.352
BB 1	28.36	1.01	81.0	0.870
BS 1	....	....	74.8	0.168
BR 4	....	....	63.4	0.334
Average	29.83	1.30	67.26	0.488
SILICEOUS SAND				
Regions A and D:				
MI 1	7.17	0.14	4.00	0.11
MI 3	9.19	0.20	4.16	0.08
MI 4	4.18	0.05	1.44	0.08
TC 1	8.37	0.18	11.54	0.03
SB 9	14.09	0.94	19.04	0.09
PG 1	12.18	1.92	16.00	0.06
Average	9.21	0.57	9.36	0.08

fibrous peat soils. The small amount of total nitrogen in most of the soils indicates a small amount of decomposition. Some of the chemical characteristics will be considered with each type of soil.

Peat Soils

Fibrous and plastic peat soils, as well as muck soils, cover any type of basic soil or substratum and occur in nearly every zone of the swamps. Fibrous peat has a characteristic morphology. It is coarse but firm, consisting of an interlocking network of many fine rootlets and pithy parts of the larger roots. Peat derived from *Rhizophora* can be distinguished by the abundance of the reddish-brown pithy roots in various stages of decomposition, and the network of finer, yellower feeding rootlets or their remains. The remains of *Avicennia* and *Laguncularia* roots are less distinctive but can be distinguished and the origin of the peat determined. Near the surface there are visible remains of leaves and small parts of stems of mangroves and associated plants in various stages of decomposition. The whole is very friable when dry. Fine-particled plastic materials occur in this fibrous network, and when abundant form a plastic type of peat which may be in definite layers. In many instances a whole profile, even to a depth of 7 or 8 feet, may be so poorly differentiated that no horizons can be distinguished. In general the peat profiles are more fibrous in their upper parts, becoming more plastic toward the bottom.

Peat generally contains little mineral material and is nearly free of woody fragments. Shell fragments, parts of calcareous algae, spicules, and other remains of invertebrates are common. These marine materials of organic origin accumulate with the swamp plant remains. The peats can be identified as to origin by microscopic examination, and in part by chemical analysis. In general the reddish-brown color of *Rhizophora* peat is distinctive, and the *Avicennia* peat is darker with more yellow rootlets and greater abundance of plastic materials. (See "The Soil Profiles and Their Significance.")

The hydrogen-ion concentration of these peats is interesting in that in general the wet peat has a pH range from about neutral to 8.1, and the dry peat from about 4.5 to 6.5.

In contrast with fibrous peat, the plastic peat of sedimentary origin becomes hard and dense when dry and breaks with a fairly smooth fracture. It usually accumulates in the quieter-water parts of the swamps, especially around lakes and ponds and on the inner margins of the swamps next to the marshes. This form of peat is derived from many materials that have decomposed more finely than the fibrous peat materials. Much of it has been washed into the swamps from the interior, or from the sea. In general it is a grayish-black, soft, oozy mixture.

Besides peat, other soils with a high percentage of organic matter may be recognized, but are difficult to distinguish. Muck soils prevail in fresh- and slightly brackish-water marshes. Besides these, the humus soils of the drier parts of the swamps and adjacent types of vegetation are also of high organic content.

The hammocks, some dunes, and some of the pinelands have such soils. The Conocarpus transition associes is frequently on marl soils with a high humus content.

In general, plant growth and decomposition are rapid in fresh-water marshes; in saline marshes both are slow; but in brackish-water marshes growth is rapid, whereas decomposition is retarded. Therefore, mangroves have excellent growth and relatively slow decomposition in most of the swamp zones. This accounts for the abundance of peat of all types in the swamps.

The chemical analyses of twelve samples of peat soils given in table 8 show an average loss on ignition of 65.2 per cent, organic carbon 7.14 per cent, and total nitrogen 1.38 per cent. These data indicate the high organic content of these soils and the probability of little decomposition of the plant remains. Although the calcium carbonate content averages high, 7.1 per cent, it varies so much from place to place that its presence is not particularly significant except as indicating marl muds associated with the peat.

The depth of these peat soils is so great in some places that they not only indicate conditions of accretion, possible changes in the sea level, and other things that will be considered later, but may also prove significant for paleontological correlations, and may even indicate the character of source beds of coal and oil.

Calcareous Soils

The shallow-water deposits of the basins in southern Florida are dominantly calcareous. The variability of the currents among the keys and parts of the indented coast sorts out the materials of different sizes and weights, bringing about a variety of sediments on the floor of the bays and sounds and in the mangrove swamps. If these sediments are of fine particles of silt and clay, and of high calcium carbonate content, they are marl muds. If the sediments are coarse, the soils are calcareous sands. The two may be so intermingled that no particular classification applies. Where there is nearly constant sorting of materials by strong currents the coarser sands predominate, and where there are protected areas of nearly uniform depth the muds predominate. As mangroves grow best in the latter situations, they are more abundant on marl muds than on calcareous sands.

Thorp (1935) has made a thorough study of these shallow-water deposits. In his average of 50 samples from stations along the Florida Keys in or near mangrove areas, the order of abundance of materials was: calcareous algae, mollusks, silt, coral, foraminifera, clay, calcium carbonate, spicules, minerals, crustaceans, and worm tubes. The remains of the organisms average over 90 per cent calcium carbonate. The sparsity of mineral grains other than those composed of calcium carbonate is striking. It seems that these many types of calcareous sediment are nearly all derived from marine organisms directly or indirectly. Some of the sediments are marl material washed from the marl uplands of southern Florida, and some of this soil is derived from the Miami oölite.

These calcareous sediments are the best soils for the initial stages of mangrove growth in Florida. Most of the pioneer communities of any size and vigor occur on shoals of marl mud ooze. The other communities of the swamps may also occur on marl muds. The firm, heavy marl of the partly submerged or emerged zones of the swamps is probably of recent marine origin, and is found over many of the inner parts of the Avicennia communities and the Conocarpus transition associes. Humus of mangrove and other origin accumulates with this marl clay until it becomes granular and loamy and supports the vegetation of the climax coastal hammock forests.

The coastal soils from Miami to Cape Sable are dominantly of this marl. The marl prairies are on nearly pure marl of the elevated portions of this zone. The hammocks are of loamy marl and humus, and the marshes and mangroves are of all sorts of mixtures of marl with fibrous and sedimentary peat materials. In some of the swamp profiles, as for example at station KL 3, there are layers of nearly pure marl between layers of plastic or fibrous peat, and this layering is very significant in interpreting the geologic rôle of mangroves.

It is not difficult to distinguish the physical and morphological features of pure and nearly pure marl soils, for they are of a grayish color and a clay texture, very slick when wet and very hard when dry. The various mixtures of marl with peats grade from soils that are slightly darker than pure marls to those that are nearly as reddish brown as the fibrous peats. The enmeshed roots of the mangroves ramify through marl soils much as they do through the peat. Only chemical analyses furnish a good basis for determining the predominance of either type when they are thoroughly mixed. As shown in table 8, a typical mixture of marl and peat at station FB 12 has 28 per cent loss on ignition and 2.74 per cent organic carbon, indicating peat, but it also contains 62 per cent calcium carbonate, indicating marl. Marl is not confined to the southern coast, for there is some in the great mangrove forest region of the southwest Everglades coast. The mesic hammocks of these swamps and their inner portions near the marshes are predominantly marl, but with more shell and sand than is common on the southern coast.

Sand Soils

The sandy soils of the coast north of the Everglades on both sides of the peninsula are dominantly siliceous except for the many shell fragments and calcareous materials of the bars and shoals of the islands and outer coast. These latter are dominantly of calcareous sands, as are the Sand Keys and the strand shores of the Florida Keys. The silicon sands correspond in each locality to the upland sand soils near them, of which many types have been described and named. Fibrous peat may form directly on top of these sands without much mixture of the two, but in most cases the mangrove soils are a sandy muck of plastic peat, sand, and some fibrous root materials. These organic sand soils are dark-colored even when not containing fibrous materials, and have a high content of organic carbon, over 8 per cent in many instances.

Offshore sands in quiet, shallow waters are frequently mixed with sedimentary muds. Upon these, pioneers of the mangrove prisere begin. Hard-packed or shifting sands, where strong currents or waves exist, do not furnish good anchorage for the seedlings. Beach sands are in general too coarse or shifting for more than a temporary growth of mangroves. In general either siliceous or calcareous sands must be in relatively quiet waters or mixed with appreciable quantities of finer materials for mangrove growth. This condition exists in many of the quieter-water bays or lagoons and in the interior of the swamps. Sands are washed into the swamps with high-water waves and settle out, occasionally forming strata between layers of organic materials.

In summary: the soils of regions A and D (fig. 1) are siliceous sands with little peat; of region B, marls with great quantities of peat; of region C, a mixture of sands and marls with even greater quantities of peat; and of region E, calcareous sands and some marl. Regions B, C, and D have some calcareous muds. Since silicon sand soils increase northward along the peninsula and mangroves decrease, it might be inferred that mangroves are less vigorous on such sands, but the lower winter temperature northward is the main cause of the decrease. The sandy nature of the soils may be only a secondary factor.

Rhizophora communities prosper on nearly all types of soil and substratum but are rare on any but wet soils. The young plants can grow among the coarsest shell and coral fragments. The largest and best growth is on deep peat soils. The fastest growth, with trees producing many seeds, is on marl soils. Avicennia and salt-marsh communities do well on all soils, including some very dry and salty near the surface. The white mangrove seems to do best on either type of sandy soil. Buttonwoods are seldom found on deep peat soils, but grow well on coarse dune sands or hard marl flats, and on relatively dry soils.

Sand is most easily washed away from the roots, marl next easily, and peat least easily; owing to this relative stability of peat, a community on peat seems to be the type that persists longest. Some mangroves on peat over coral rock occur along the exposed shore of the Florida Keys, having persisted there for a long time, as indicated by their deep peat soils. Such is the case at station KL 1, where the raw fibrous peat is 5 feet deep along the ocean front.

Water Content of the Soils

The water content was determined for nearly all types of soil, and is given in table 9. The percentage of water was determined by comparing the weight of the soil samples soon after being obtained and when oven-dried. The data show that the peat soils hold the largest quantity of water, although they dry more rapidly than marl soils. The coarse calcareous sands hold the smallest amount of water, but most of it is readily available to the plants.

Many of the surface soils of the inner zones of the swamps are exposed, between tide floodings, to periods of drying which in some cases are very long, as some soils are wet only by the highest spring tides. Nevertheless, it seems that soil dryness is seldom a limiting factor for mangrove growth. Even though the amount of water in calcareous soils is small, the roots of the mangroves can

TABLE 9. *Water content of different types of soil at selected stations*

(In percentage of the oven-dry weight of the soil)

Stations	Soil	Percentage of water
	PEAT AND MUCK SOILS	
KL 1	Fibrous peat	297.3
KL 3	Fibrous peat and marl	117.5
KL 4	Fibrous peat	212.4
BB 1	Fibrous peat and marl	135.5
CS 1	Fibrous peat	320.5
BR 1	Fibrous peat	287.4
EC 11	Sandy fibrous peat	136.5
LB 3	Sandy fibrous peat	171.6
CR 3	Plastic peat muck	128.3
CS 4	Mangrove peat and hammock humus	183.3
Average		198.0
	MARL SOILS	
KL 6	Marl mud ooze	192.0
KL 2	Marl top layer swamp	164.2
FB 6	Heavy marl flats	92.4
FB 8	Hammock marl	127.2
CS 6	Heavy marl	68.0
BB 1	Marl top of peat	94.3
Average		123.0
	SAND SOILS	
BK 2	Calcareous sandy soil muck	58.5
BK 1	Calcareous strand dune	12.2
LK 1	Coral ballast and sand	8.3
KW 6	Calcareous hammock	31.0
MI 1	Siliceous sand and marl	81.2
CR 2	Siliceous sand muck	68.1
CR 4	Siliceous sand muck	76.0
SP 1	Siliceous sand muck	58.1
Average		47.0

readily penetrate such loose soils to the water table. In some cases the soils of the scrub mangrove facies and the salt-marsh flats may lack sufficient water in the soil to maintain plant growth, but in most mangrove communities the soils are nearly always saturated. In general, water content increases at lower levels of the swamp and with increase in organic matter.

Tides, Water Level, Aeration, and Drainage

This set of factors are interactive and have much to do with the zonation of vegetation in mangrove swamps. Of these the tide is less important than might be supposed, because there are numerous tideless or nearly tideless parts of the Florida coast that have the same or even better zonation of mangrove communities than do some places with regular tide. The water level with or without tidal influence is the most important of this set of factors. Aeration of the submerged parts of the mangroves is important and is accomplished by the aerenchymous tissue of the mangroves. Drainage from the interior is important in some places and at certain times.

The Tides

The irregular coasts with abundant offshore islands, bars, and many shoals cause a great tidal irregularity. Along the outer, deep-water side of the coasts or the Florida Keys toward the Straits of Florida the tide may be regular, but in the bays and sounds and along the very indented parts of the coast the tide may be very irregular or nonexistent. Prevailing winds may force water from one shallow-water basin to another against the normal direction of the tide. For these reasons it is difficult to make any general observations about tides, and the effects of tidal range at any one locality can be determined only by study at that place.

The tide tables of the U. S. Coast and Geodetic Survey give data on the tide at many places in Florida. The averages for the mean tidal ranges of some of these regions are: (1) the Atlantic coast from Cape Canaveral to Miami, 2.6 feet; (2) the Indian River, 0.9 feet; (3) the Straits of Florida side of the Florida Keys from Miami to Key West, 1.9 feet; (4) the Sand Keys from Key West to the Tortugas Keys, 1.2 feet; (5) Barnes Sound between Key Largo and the mainland, 0.2 feet; (6) Florida Bay, 0.5 feet; (7) Cape Sable, 2.9 feet; (8) from Shark River to Cape Romano along the southwest coast, 3.3 feet; (9) the west coast from Lemon Bay to Cedar Keys, 2.1 feet; (10) Tampa Bay, 1.5 feet. The highest spring tide is only 4.5 feet, at the mouth of the Shark River. In the eastern part of Florida Bay the periodic tide is negligible. In Blackwater and Buttonwood sounds there is seldom any tide, and many of the more inland brackish-water lakes, as Cuthbert and West lakes, have no tide.

If mangroves grew only in the intertide zone, they would cover very narrow horizontal zones even along these low-lying coasts. This, however, is not the case. The Rhizophora communities begin in water almost constantly 1 to 2 feet deep and extend to levels that may be flooded by only a few tides during a month. The Avicennia communities range from a zone nearly always flooded by a few inches of water to levels flooded only by the highest spring or storm tides. The Conocarpus transition zone is seldom flooded by any but storm tides or unusually high spring tides. Many communities back from the coast are never flooded by tides.

Figures 2 and 3 illustrate the general relation between tidal levels and the communities, but very often the same zonation occurs without any tide. Along the southern coast zonation without tide is about as frequent as that related to tide, and in many cases is even sharper than where there is a great tidal range. Thus, the zonation in and around Florida Bay is more definite than that of the mature mangrove forest of the southwest coast, where the tide averages 3 feet. Figure 4 shows the zones near West Lake, where there is no tide, and illustrates conditions near the southern tideless or nearly tideless coast.

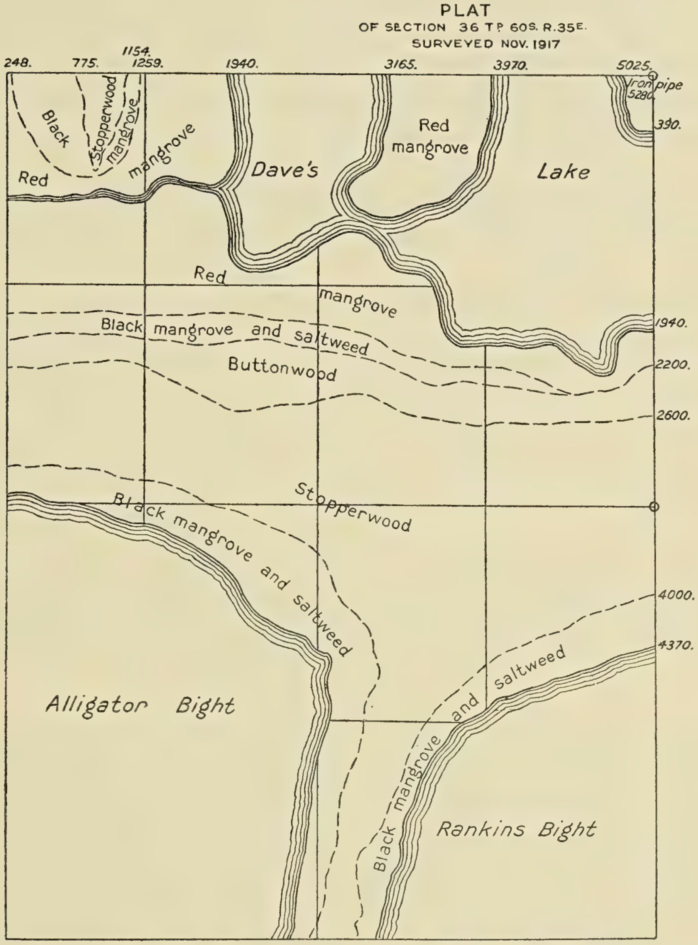

Fig. 4. Copy of plat surveyed by A. R. Livingston in 1917, showing definite zonation of mangrove and associated vegetation.

Many students of salt marshes (Chapman, 1938; Johnson and York, 1915) and also of mangroves (Watson, 1928) correlate the distribution of species with the frequency of tide inundation. In the Malayan forest Watson found it possible to make rough correlations between the types of vegetation and five tidal classes based on their height. In that region (data from Port Swettenham), the average tidal range is 6 feet, which is much greater than that in Florida. Also Watson recognized that "these divisions are entirely arbitrary" and the arrangement of species by inundation classes gives a key only to the most typical distribution and fails to include the whole range of the species.

Exposure and drying between high tides may harm young mangroves and is important in some of the inner zones of the swamps. Unusually low neap tides in some places drain off 4 to 6 inches of water from the level of the normal low tide. In this way wide areas are exposed that are normally flooded. If this low-water condition lasts for any length of time the surface soils become dry, but few instances were found of any appreciable harm to the plants. The interval between these low tides and the next high tide is seldom more than a few hours. The soils hold enough water (table 9) to supply the plants during the exposure interval.

In many places, particularly on the Ten Thousand Islands of the southwest coast, the normal low tides expose the soils of the mature Rhizophora consocies and some of the pioneer Rhizophora communities. The algae thus exposed seem unaffected by the short interval, and the mangroves show no ill effects, except for the very young seedlings that have not taken good root. Only under conditions like those found in the "salt flats," where there is heavy marl mud over level flats just above the normal tide level, are the soils too dry for normal plant growth.

The tide is of major importance in the soil-accretion processes and in the dispersal of seedlings, which are considered later. Tides cause the exchange of water which is so important in salinity, aeration, and to some extent drainage.

Water Level

The water level above or below the soil, and with or without the direct influence of the tide, probably has more influence upon plant zonation in the swamps than any other factor. In the discussion of the plant communities this factor has been stressed. Although no uniform condition of height of surface water or depth of water table below the soil exists in every similar plant community, there is a more or less definite correlation between water level and the type of plant community. Many measurements were taken to determine average water levels in the different communities, and the results given in table 10 are fairly representative. It is, however, difficult to determine average water-level conditions because of the many changes due to seasons and tides. Since most of the field work was done in the summer, the data are not representative of conditions in the drier winter months. It was difficult to penetrate some swamps at high tide, and many of the measurements of water level below the soil surface were slightly inaccurate because of inability to make measurements during all conditions of tide; most measurements were made at low tide. It is in general true, however, that there are progressively lower water levels above the soil from the pioneer Rhizophora families to the Conocarpus transition associes, with surface water rare over the Conocarpus zone and the inner part of the Avicennia salt-marsh associes, but almost constantly present over the Rhizophora communities.

A difference of a few inches in depth of the average low water table below the surface has a marked effect upon much of the inland vegetation adjacent to mangrove swamps, but it is seldom a critical factor in the swamps. Only in the rarely flooded inner zone of black mangroves and buttonwood is it probable that the roots fail to reach saturated soils at all times. Even then the soils, except coarse sands, have a great water-holding capacity and provide sufficient water, as is shown in table 9. Capillarity from the saturated level, at most a few inches below the surface, keeps all but the very crust of these soils damp. As *Avicennia, Laguncularia,* and *Conocarpus* have shallow root systems, they are dependent upon a near-surface water table, which is in most instances present.

TABLE 10. *Average water levels in the mangrove communities*

Community	Height of surface water (inches)	Depth of average low water table (inches)
Pioneer Rhizophora family	16	....
Mature Rhizophora consocies	10	....
Avicennia salt-marsh associes	6	....
Conocarpus transition associes	....	4
Scrub mangrove facies:		
In paludal basins	5	....
On salt flats	....	3
Salt-marsh associes	....	2

Aeration and Drainage

The prevalent condition of submergence, particularly where the surface water is stagnant without much tidal exchange, leads to conditions of poor aeration for the under-water parts of the mangroves. The oxygen content of the water was not measured, but is assumed from the degree of stagnation. Both *Rhizophora* and *Avicennia* are well provided with aerenchymous tissues and "breathing" organs. *Rhizophora* is equipped with pithy lenticulate arching prop roots, some part of which nearly always remains above the highest water. *Avicennia* produces pneumatophores sufficiently high to reach above the average highest water level. The careful measurements by Karsten (1891) of *Bruguiera eriopetala* pneumatophores, and by Schenck (1889) of *Avicennia tomentosa* and *Laguncularia racemosa* to determine the function of roots as respiratory organs, show the need of these organs in the saturated and poorly oxygenated muds of these swamps. These organs help maintain an adequate gas exchange under the fluctuating conditions produced by tides. Suffocation due to lack of soil and water aeration is indicated in the paludal basins that have little exchange of surface water. In such places mangroves are usually dwarfed and form a scrub mangrove facies. Interference with proper drainage creates unfavorable conditions in many zones of the swamp. In general, better drainage and aeration are associated with Rhizophora communities, and *Laguncularia* and *Avicennia* tolerate the poorest drainage and aeration conditions.

Since a heavy rain may produce the mechanical, if not the physiological, effects of flooding, the surface water may be a limiting factor without any apparent relation to its salinity or tidal fluctuation. The brackish-water and fresh-water marshes become deeply flooded during the summer rains, and from these large quantities of water drain over the mangrove swamps. The seasonal drainage from the vast Everglades marshes is slow owing to the very flat topography, and this water may stand over the inner zones of the swamps for a long time because of the poor neap tides. Dilution of the salt water after these rains has been measured and is great in many instances, but the effects of the increase in height of the surface water and other changes have not been measured, for they seem of little importance. Seldom is drainage severe enough to cause a flood that washes away soil or mechanically injures the trees. The high water aids in washing in organic débris, plastic peat, and sediments, and to some extent in the dispersal of the seedlings.

CLIMATE

Temperature

Minimum temperatures are of primary importance, as mangroves do not survive hard frosts. The minimum temperature they will survive has not been determined, but it seems to be below 32° F., as U. S. Weather Bureau stations near mangrove areas have recorded lower temperatures without much injury to the mangroves. Temperatures of 27° at Miami in 1917 and 30° at Flamingo, Cape Sable area, in 1905 apparently did not kill the trees, as many of them near these places seem to date from before these frosts. The freezes at Merritt's Island (region A, fig. 1) in 1894–1895 reached a low of 22°, and according to residents killed all the mangrove trees. At Fort Myers during the same season temperatures of about 20° killed the mangroves in adjacent swamps. It seems, then, that air temperatures below 25° kill the trees, but the exact temperatures of the water in these instances were not found in the literature. Legend has it that a great frost in 1825 killed all the tropical vegetation to Key West, but this is undoubtedly false, as some of the large red and black mangrove trees in the forests of the southwest coast region seem to be more than 114 years old.

Some of the above data are taken from Mitchell and Ensign (1928). They show that the coastal areas which are usually free from the occasional frost that visits the inland regions of Florida are also the areas that are covered by mangroves. Undoubtedly mangroves survive when temperatures are recorded as freezing at inland stations. The upland plants near the stations may be killed, yet mangroves, because of their position near water, may survive. Storey and Gudger (1936) have given an account of some of the effects of low temperatures in the mangrove areas, and show that the temperature of shoal water falls nearly to that of the air. No doubt low temperatures are effective in limiting growth as far south as Fort Myers on the west coast and Jupiter on the east coast, as few large mature mangrove swamps occur north of these places.

A few mangroves occur in Louisiana and other parts of the Gulf of Mexico coast well beyond the subtropical and tropical regions, and except for occasional unusual low temperatures mangroves would be more extensive in Florida than they now are. They are so rarely associated with south temperate types of shore vegetation that it is obvious that they definitely do best under tropical or subtropical conditions.

Rainfall

Rainfall varies a great deal from season to season and place to place along the Florida coast, but these conditions do not affect mangroves directly because their roots are almost constantly in saturated soils and their aerial parts are protected against excessive water loss. No significant correlations between mangrove growth and annual and seasonal precipitation were found. Thus, although the rainfall around Miami is much greater than that of the southwest coast across the peninsula, the mangroves are unaffected by this difference.

Excessively long dry spells, such as occurred in the winter and spring of 1938, may cause a lowering of the inland water table and surface water levels such that the inner zones of the swamps become too dry or too salty for normal growth. Many plants of the Conocarpus transition associes shed their leaves from the effects of such droughts. Frequently, too, these dry areas may be burned by fires spreading from the interior. Such burns have killed many mangroves along the margins of the Everglades in recent years, particularly since the excessive drainage brought about by digging canals.

The secondary effects of rainfall, particularly seasonal rainfall, have been pointed out in the sections on salinity, drainage, and other factors. The heavy summer rainfall related to topography is important, causing the flooding of the marshes and swamps with fresh water and the drainage of great quantities of this water into the shallow-water basins.

Storms

Storms are of common occurrence along all the coasts. Some are not violent enough to disturb the mangroves, but the occasional tropical hurricanes may be very destructive. Those of 1926 and 1929 in the Miami region, and 1935 across the Florida Keys and Cape Sable, completely killed most of the trees in the center of the storm track. In a severe hurricane, the trees, because of their strong anchoring roots, are not all uprooted, but their trunks and limbs are so violently shaken that the bark is loosened from them and falls off. Salt spray and sea waves are swept into and through the swamps, and batter all leaves and nearly all branches off the plants even if the winds do not shake them to death.

A few of the smaller, better-protected mangroves may survive even the worst blows, and buttonwoods survive best of any tree of these swamps. From a few plant survivors and from seedlings or seeds washed into the swamp a new forest soon starts, except where the storm sweeps away soil so deeply that mangroves cannot begin growth. Some smaller islands and exposed shores have been thus completely destroyed. Such erosion would be very much more intense at any place, and much more extensive, were it not for mangrove growth. In this respect these swamps are effective conservers of the coast and islands.

The sediments and débris swept in by these storms may contribute a great deal to elevating the soil level. In some cases low and young growth may be covered up and killed. The water washed over the swamps does mechanical damage but soon drains off. Such unusually high water, and the spray which is blown many miles inland, are effective in extending salinity well beyond the normal range of the tides.

Evaporation and Transpiration

The evaporating power of the air in this tropical region is intense, and without adequate control of water loss the plants would be harmed by excessive transpiration in such a saline habitat. The evaporating power of the air was measured at representative places by means of standardized Livingston porous-cup atmometers, and averaged 20.48 cc. per day for the mature Rhizophora consocies and 21.56 cc. per day for the Avicennia salt-marsh associes. At one station in the open, where winds had full play, the rate was slightly higher, 25.45 cc. per day. These values are much higher than those obtained for evaporation on exposed mountain peaks at 6000 feet elevation, where evaporation is considered intense (J. H. Davis, 1929).

The xeromorphic nature of mangrove leaves (pl. 11, figs. 1, 2), which partly protects them from excessive water loss, has been studied by a number of persons, and Bowman (1916, 1917) very thoroughly demonstrated the differences of structure of *Rhizophora* leaves in water of different salinities. Some similar observations on *Avicennia, Laguncularia,* and *Conocarpus* as well as *Rhizophora* have been and are being made with plants growing both in their natural habitat and in experimental cultures. The results so far indicate that transpiration rates are slower the higher the salt concentration, but that they also vary with the character of the soil if the atmospheric humidity, temperature, and wind are the same. A very complicated physiological relation exists, with which we are not primarily concerned here.

The strongly developed xeromorphic character of the leaves (Arzt, 1936), in spite of the fact that their environment is aquatic, and the peculiar organs for respiration are, without doubt, of ecological as well as physiological and morphological significance, but this paper cannot cover all such relations. Xeromorphism is considered by some persons as indicative of the "physiological dryness" of the habitat, but such a concept does not seem adequately to explain the condition in mangrove swamps.

Biota

The number of animals, as well as plants, commonly associated with mangroves is large. The inundated forest has a highly specialized marine fauna, including many invertebrates and, in some parts of the world, such curious fish as *Periophthalmus.* Miner (1937) and Simpson (1932) give popular accounts of some elements of this fauna. A few of the most interesting forms were observed and collected and some of their effects on the plants and soils noted. In addition to the strictly marine animals, there are shore and littoral forms and strictly land forms. These include reptiles—turtles, crocodiles, alligators; mammals—bear, wildcat, puma, rats; many water birds; myriads of crustaceans; and abundant mollusks. The mammals, reptiles, and most of the fish are of little significance. The mollusks, crustaceans, and birds are most important.

Among the mollusks the coon oysters, *Ostrea frons* (pl. 3, fig. 2), which in some places encase the red mangrove roots, most definitely affect the growth of the plants. Some stilt roots bearing them are injured and finally rot, or break off with the increased load. A number of gastropods such as *Cerithium, Litorina, Cypraea,* and *Melongena* are found on the mud flats and crawling on the roots and stems. Some of these form a series successively more independent of the sea water, entering brackish water or becoming terrestrial. The periwinkle *Litorina irrorata* seems to be on the way from the sea out to the land. Many other bivalves besides the oyster live on the sandy or muddy flats, or on the mangrove roots. The effect of most of these mollusks is to increase the amount of shell débris accumulating in the swamps.

Fiddler crabs, *Uca,* and many other crabs swarm over the sands and muds of the swamps and adjacent beaches. The effects of burrowing by some of these animals in the wet soils may be good for aeration and mixing of the soil. Damage to the roots and young plants seems to be negligible.

The burrowing worms, brittle stars, jellyfish, sponges, and other tidal or shoal-water invertebrates are of little direct influence on mangroves. Some are very common in a particular part of the swamp, for example the jellyfish *Cassiopea* in the Avicennia salt-marsh associes. The relations may be purely casual or definitely obligatory. No doubt some of these would be worth detailed study.

A few characteristics of mangroves seem to have an effect on the animals. One of these is that tannic acid seems to prevent the boring of the shipworm, *Teredo.* The holes found through the stilt roots of *Rhizophora* are credited by some persons to this animal, but none were found in the holes; instead, an unidentified burrowing crustacean was nearly always found, and may have caused the damage. Tannic acid also affects the water in the swamps so much that it becomes unsuited to some animals. Obviously the changes in oxygen content, salinity, alkalinity, carbon dioxide, and other chemical relations affect the animal life.

Barnacles attach themselves to the young seedlings, but they seldom incrust the roots or stems sufficiently to do any noticeable harm. Myriads of small crustaceans and protozoans thrive in the plankton and soils of the swamps. Locally foraminiferan shells are abundant in the bottom deposits.

Associated with this marine animal life are marine algae, some of which are calcareous and contribute much to the bottom deposits. These algae, the marine seed plants, and the shoal-water animals are competitors with mangroves of the pioneer communities. There are few bare shoals, so that mangroves, mostly *Rhizophora,* must be able to invade, grow, and establish themselves against severe competition. (See "Dispersal and Establishment.")

Many kinds of water birds, both local and migratory, use the mangrove bushes and trees for rookeries or roosts. Some of the islands and coastal swamps are so famous for their bird life that they bear the name of a bird or are known as a certain rookery. The pelicans, egrets, herons, and other large birds which roost or make their nests in the trees may do damage to the foliage and limbs. If the population of birds is large for the area, there is some injury and even destruction of the vegetation. This effect is local and of little general importance.

The excreta from the birds accumulate in the swamps, causing a high nitrogen content of the soil, but no apparent harm or good to the vegetation has been noticed.

Why the birds inhabit one particular island or locality along the coast to the neglect of others, and why they suddenly forsake a long-established place, was not determined. Such action does not seem related to the vegetation, unless it is reduced or destroyed, but rather to the food supply.

DISPERSAL AND ESTABLISHMENT; PLANTINGS

Introduction

Few plants illustrate the effects of ocean currents on dispersal as well as do mangroves. The Eastern mangroves of India and Malaya and the east African coast have no species in common with the Western mangroves of the west African and American coasts. All Florida species are found on both tropical coasts of the Americas and on that of west Africa. The plants probably migrated to the Americas from Africa by the equatorial current. How they got to the west coast of the Americas is uncertain; it is improbable that they migrated around Cape Horn, since they do not migrate around the Cape of Good Hope, as evidenced by the difference in the species of the two African coasts.

The eastern flora is rich, the western flora poor, and the only indication of mixing is in the Pacific. Guppy (1906) found no mangroves in Hawaii, but both the eastern *Rhizophora mucronata* and the western *Rhizophora mangle* have been reported there recently. One or both of these species have been planted along the coast, and some persons think they may have migrated there also. Guppy also reported finding both these species in Fiji and Tonga and suggested that the present distribution of *Rhizophora mangle* is representative of its original range over the tropic zone. Ridley (1930) considers Guppy's conclusion improbable, thinking it more likely that *Rhizophora mangle* spread across the

Pacific by successive stages of islands from the American coast 6000 miles distant. Such may be the case, as very distant dispersal seems probable from these Florida studies of rate of floating and the long duration of viability of the seedlings, especially of *Rhizophora.* Ridley reports that the seedlings drift to the coral reefs of Cocos-Keeling and Christmas islands in large numbers and some have lodged on the shores of Krakatao since its eruption. A thorough study of the world distribution of mangroves would be instructive as to both the plants and the currents.

The Florida situation afforded a good opportunity to study local dispersal and determine the main factors involved. The complete or partial vivipary of the seedlings, their mode of floating, and the length of time they can float without harm make these plants uniquely adapted to migration by water. The three species were studied in as much detail as the seasons of work would allow.

The nature of the currents from Key West toward the Tortugas Keys furnished a fine basis for an experimental study. A brief consideration of some of the field and experimental observations on dispersion; the currents and other factors most related to dispersion; the establishment of the plants; and some of the results of experimental plantings is presented here.

Dispersal of Rhizophora mangle

Many seedlings of *Rhizophora* were collected during June, July, and August, when most of them mature, and after being marked with different-colored paints were cast overboard at selected places. The best results were obtained from those thrown overboard at intervals along the course from Key West to the Tortugas Keys. Some of these marked seedlings were collected on the beaches or in the water as they were being brought to the shore by currents. In two instances a sufficient number were recovered at the Tortugas when they were just arriving on the beach so that the data are reliable. The direction of the currents was indicated by these data as well as by measurements of the currents. Table 11 gives the results of this experiment, and figure 5 indicates the location where seedlings were cast off and their probable route to the islands, also the probable route taken by the seedlings that missed the islands. Considering the

Table 11. *Results of experiments to determine rate of travel and number recovered of marked Rhizophora mangle seedlings cast overboard between Key West and the Tortugas Keys, summer of 1938*

Location where cast overboard (fig. 5)	Distance from Tortugas (nautical mi.)	No. cast overboard	No. recovered	Rate of travel (knots)
A.	38–40	1500	0	. . . .
B.	26–35	1000	0	. . . .
C.	15–20	500	19	0.38
D.	7–10	1300	21	0.17

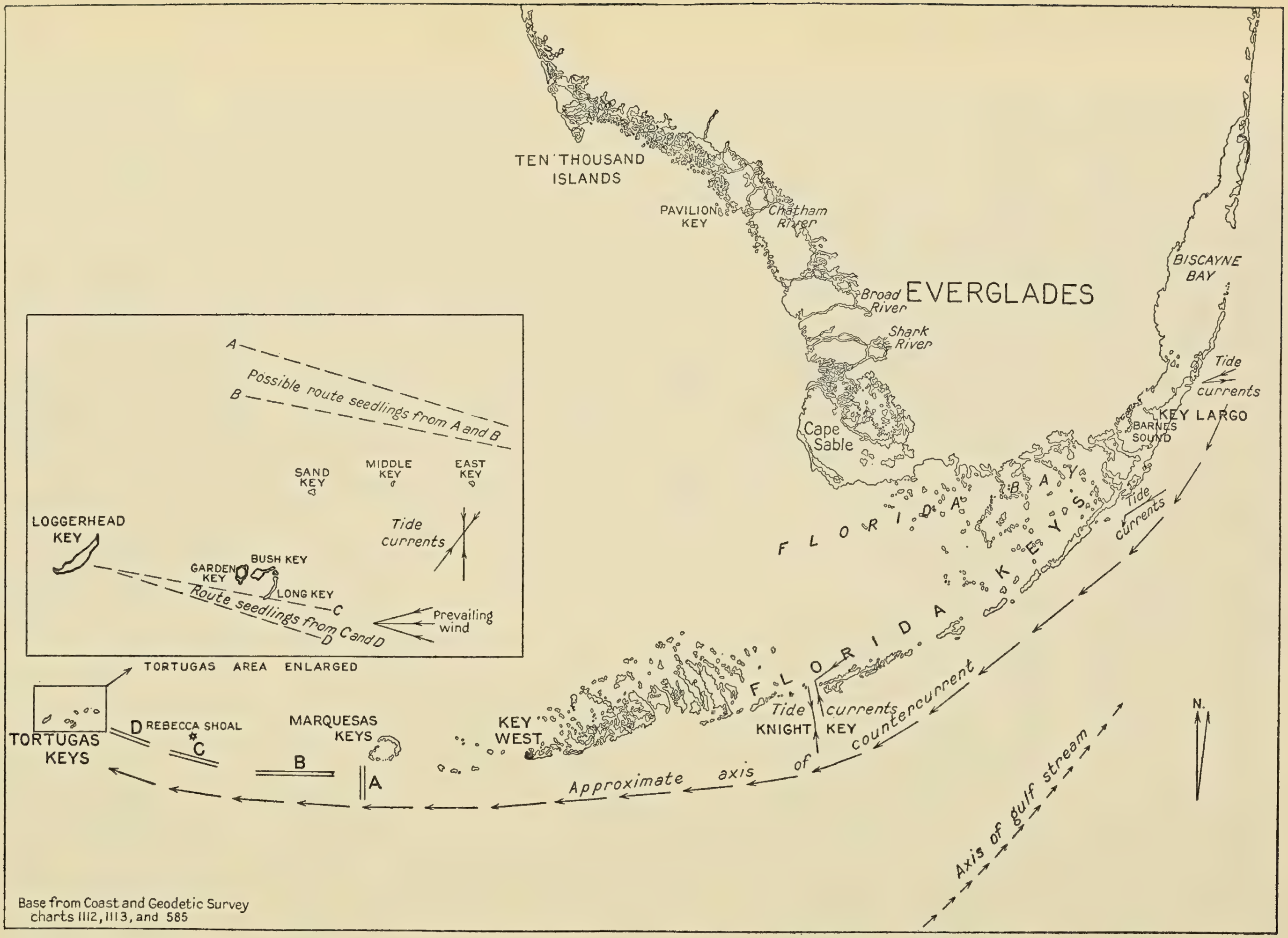

FIG. 5. Map of southern Florida and the Florida Keys, showing: counter current; tide currents at four places; prevailing winds at the Tortugas Keys; dispersal of *Rhizophora mangle* seedlings cast overboard at positions A, B, C, and D between the Marquesas Keys and the Tortugas Keys, with probable routes of floating. (Length of arrows indicating direction of tide currents is proportional to velocity of ebb and flow; see table 15.)

short shore length of the islands of the Tortugas, and the fact that most of these seedlings were found along about 1000 yards of beach on the east side of Loggerhead Key, the small number recovered is a very high percentage, indicating that the plants do not fan out very widely but follow a fairly narrow route.

The countercurrent that flows along the outside of the Florida Keys to Key West continues to the Tortugas and is the main cause of the direction of floating of the seedlings in this experiment. The prevailing winds are also from the east and help transport the plants westward. There is a definite nearly north-south and south-north tidal current along most of this route, with the surface south-north flow stronger than the ebb, so that the seedlings are shunted slightly northward. Vaughan (1935) gives accurate data on most of these currents, and his figures show a definite mixture of currents within or near the Tortugas atoll, so that the seedlings are probably more scattered near these keys than along any other part of their route.

Some very instructive data were also secured from collections of seedlings on the Tortugas Keys. Since none of the mangroves on these islands grow near the water's edge, all but the marked seedlings collected along the beach were brought in by currents, which are probably all from the Florida Keys or the mainland. Collections were made during one 10-day period in 1937 and two 10-day periods in 1938 (pl. 9). As few seedlings survive on the sandy beaches from one year to the next, or last for more than a year in sufficiently good state of preservation to be recognizable, the collections each year probably indicate the number arriving that year or part of a year. Obviously the number found is only a part of the total number. Table 12 summarizes the results of these collections. After some experience the seedlings could be classified into those that had arrived recently, within a month or two, and those that had arrived earlier, so that the total number of recent arrivals collected can be given. Whatever their origin, seedlings must be floating in the sea in great quantities directed by currents toward these islands, for it is very probable that only a small percentage

TABLE 12. *Collections of Rhizophora mangle seedlings migrating normally to the Tortugas Keys*

Island	Collected or observed	
	1937	1938
Loggerhead Key	174	183
Bush Key	187	176
Long Key	18	16
Garden Key	47	24
Sand Key	36	62
East Key	23	38
Total	485	497
Number arrived *recently* on all keys	137	128

of the total number afloat become stranded. If those collected are 5 per cent of the total afloat, which is twice as high a percentage as that recovered from experimental seedling dispersal, the total number afloat in the direction of the Tortugas in one year may be well over 10,000 seedlings. The real number is probably much greater. With numerous seedlings afloat, some should be observed from shipboard, but in six trips to and from the Tortugas only two seedlings were observed.

Bowman (1917, 1918) noted the common occurrence of *Rhizophora* seedlings washed to these islands, and Millspaugh (1907) recognized their dispersal by currents from one to another of the Sand Keys.

Seedlings of *Rhizophora* were found washed up on beaches at Jupiter, Englewood, St. Petersburg, and Clearwater on the Gulf, and at scattered intervals along the banks of the Indian River on the Atlantic coast. The large numbers found washed up high on the Gulf coast beaches after some squally blows from the west indicate the great number of seedlings afloat in the Gulf of Mexico. In one instance at St. Petersburg beach 528 of these seedlings were found along a 500-yard strip of beach.

On one occasion when 500 marked seedlings were cast overboard into Lemon Bay near Englewood, 3 were recovered on Chadwick Beach the next day, having washed out through the mouth of the bay and traveled along the coast by shore currents. Such local currents seem to account for most of the dispersal for short distances along the coast and between islands.

The time and manner of production of seedlings, their fall, the length of time they float, and their position while floating and becoming stranded are important considerations.

The long hypocotyls of *Rhizophora* develop from the fruit and to "ripeness" within 70 to 90 days (pl. 10, fig. 1). Most production of seedlings takes place during the summer months, with the largest "crops" in July and August (pl. 4, fig. 1). All stages of development, however, may be found on some trees at any time of the year. The usual height of the limbs bearing these pendent seedlings is 4 to 20 feet above the water or soil. The hypocotyls are more often curved than straight, and not many of them fall with enough force, or straight enough, to plant themselves on the spot. When they fall into water most of them are shunted off and bob up after the force of the fall is spent, to float away. Those that float out of the swamp for some distance are of importance in dispersal. The above observations were secured by dropping over 1000 seedlings from various heights into different depths of water or different types of soil.

It is particularly important to know that only a minor part of the seedlings become anchored beneath or even near the parent trees. The average vigorous red mangrove tree produces over 300 seedlings during a summer season, and probably over half of these float away from the swamp. If the swamp is flooded by the tide more float away than if it is not. There may be some hundreds of thousands of seedlings thus set adrift near the south Florida coast.

Peculiarly, no production of seedlings was found along the estuaries of the southwestern Everglades coast. The best production of seedlings seems to be by vigorous young trees, 10 to 30 years old, on marl soils, but the factors influencing seedling production were not determined.

After being set afloat the seedlings assume different positions depending upon their ripeness (pl. 10, fig. 2), the length of time afloat, and the specific gravity of the water. In general they begin floating horizontally, gradually assuming an inclined to vertical position. Salinity with its effect on specific gravity affects seedling position. Many seedlings were found as they were being washed onto the shore or into a swamp floating vertically with their epicotyl points just at or barely below the surface. In some cases the root point was scraping along the bottom.

Two sets of experimental observations were made by placing seedlings in slat-enclosed pens which permitted nearly normal wave and tide action. Over 200 seedlings were thus tested. In sea water of specific gravity 1.024 at 28° C., all but 2 per cent floated horizontally at first. After 10 days 40 per cent had inclined their root tips downward or were vertical. After 35 days all floated vertically or nearly so, and some had sunk to strike bottom. Where the depth of the water at mean or nearly low tide was the same as their length, many had begun to take root. After 60 days over 80 per cent were rooted. In brackish water, specific gravity 1.017 to 1.011 at 28° C., the results were indicative of the effect of the lighter water. From the first more than 2 per cent were inclined toward the vertical, and after 25 days all but 5 per cent floated vertically or nearly so. More sank and became rooted in a shorter time.

Even more accurate measurements of changes in buoyancy of all the parts of the seedlings were obtained by cutting them into centimeter segments and observing the floating or sinking of these segments in water of four different specific gravities. The results are given in table 13. Seedlings of uniform length, 18 cm., were chosen, and all were nearly of equal age or "ripeness." A definite difference in buoyancy of the segments from the root end toward the epicotyl end was apparent regardless of type of water, but the lightest part, toward the epicotyl end, so well balanced the heavier part of the root end that a horizontal position was assumed at first by most specimens. The differences in buoyancy of different fractions along the seedlings in different densities of water were striking. In sea water of 1.030 specific gravity at 28° C., more than twice as many segments floated as in fresh water, but the differences were not as great as might have been expected from observations by Guppy (1906) and others, who thought that most seedlings floated horizontally in sea water and vertically in fresh or nearly fresh water.

With increase in age in the water there is a tendency for seedlings to become heavier throughout, so that the number of segments that float decreases. Thus the root end is directed downward until the plants are vertical or nearly so. Many seedlings eventually become heavier than the water and sink. The cause of this gradual increase in weight was not investigated, but Bowman's (1917) studies of the enzymes of these seedlings indicate definite changes in their carbohydrate composition with age, which might in part account for their increase in weight.

The efficiency of these seedlings is obvious. When they float to a shallow-water spot where they can grow, the sharp point of the hypocotyl presses against the soil and becomes more or less embedded. If they are vertical, or nearly so, the action of the tide moves them about, forcing the root end deeper into the soil. If they are stranded horizontally, the rising tide pushes them up into a vertical position. Guppy (1906) thought that the horizontal position prevented the plumule from drying while floating and that in a vertical position the plumule withered, but this is not the case, as the tops are seldom if ever sufficiently exposed while floating to become withered, no matter what position the seedlings assume.

TABLE 13. *Average relative buoyancy of centimeter segments of Rhizophora mangle seedlings in water of different specific gravities after different intervals of time*

(Segments numbered from root tip toward epicotyl. All seedlings 18 cm. long)

Specific gravity of water at 28° C.	No. seedlings tested	Segments sinking	Segments floating
TESTED WITHIN 3 DAYS AFTER COLLECTING			
0.998	30	1–9	10–18
1.011	16	1–7	9–18
1.023	28	1–5	6–18
1.030	14	1–4	5–18
TESTED 10 DAYS AFTER COLLECTING			
0.998	40	1–9	10–18
1.011	12	1–8	9–18
1.023	36	1–6	7–18
1.030	10	1–5	6–18
TESTED 30 DAYS AFTER COLLECTING			
0.998	26	1–11	12–18
1.011	14	1–9	10–18
1.023	24	1–7	8–18
1.030	10	1–5	6–18

In addition to the above experiments, a number of seedlings were studied to observe the time and manner of origin of the secondary anchoring and absorbing roots. Some of these roots sprout quickly, after 20 to 30 days of floating, and others sprout after 3 or 4 months, the time seeming to depend upon the ripeness of the seedlings. Seedlings kept in the laboratories in water of different salinities showed no consistent time of sprouting of secondary roots, but nearly all sprouted roots, indicating that degree of salinity has little effect on sprouting.

The maximum or even average length of time these seedlings will float under normal conditions has not been properly determined. From indirect evidence, the maximum must be a year or more. Marked seedlings should be set afloat under normal conditions and recovered over a long period of time. The difficulty of doing this is obvious. The approximate length of time they will float was observed by keeping some in vessels in the laboratories. Of over 200 seedlings thus tested, many have floated or remained submerged for over 12 months without apparent harm; that is, when planted after this long period they took root and grew. Many of them developed secondary roots and began top growth while floating. *Rhizophora* seedlings make very long journeys at a relatively slow rate. Moreover, it is common to find seedlings afloat with barnacles growing on them. If the age of the barnacles could be determined, that might throw light on the subject.

The efficiency and hardiness of *Rhizophora mangle* seedlings is a striking autecological feature of this plant, and probably of related species. Not only do they float well and survive long journeys, but their upper parts do not dry out rapidly when they are once stranded by a tide that later leaves them exposed. On a beach they may survive for many months if implanted in an erect position and surrounded by plant débris which holds moisture in the soil, but in general they are not able to survive in loose, shifting soils.

Dispersal of Avicennia and Laguncularia

Besides *Rhizophora,* the Tortugas Keys have a young swamp of *Laguncularia* around a pond on Bush Key, some young plants of *Avicennia* on Bush Key, and a few old ones on Garden Key. *Conocarpus* was established on both Garden Key and Bush Key some years ago. How these species got to the Tortugas and to many of the most isolated of the Florida Keys is not certain but should be considered.

The nearly mature fruits of *Avicennia nitida* and *Laguncularia racemosa* are shown in plate 11, figures 1 and 2. The season of ripening of fruits of these two species is after August, so that in only one instance were personal observations of abundant falling of the seeds possible. A reliable assistant reported some of the observations given here. Seeds of both species were shipped from Florida to Memphis and their floating and survival in sea, brackish, and fresh water were tested.

The field observations, the laboratory tests, and the distribution of these species in the same regions with *Rhizophora mangle* indicate that both *Avicennia* and *Laguncularia* have aquivectant seedlings, being carried by the same currents that transport *Rhizophora.* Why Millspaugh (1907) considered *Avicennia* and *Laguncularia* avivectant can only be guessed, for he could hardly explain the floating habit of these seedlings and their nearly constant occurrence with *Rhizophora* by supposing that birds carry them on their feet. No report of an ornithologist who has observed this has been found.

Great numbers of *Avicennia* and fewer *Laguncularia* seeds are shed into shallow water during the fall months. These float readily in brackish as well as sea water. The hairy radicle of *Avicennia* soon sprouts, the seed coat splits off, and the folded cotyledons open. Although some of these *Avicennia* seedlings last over 4 months under laboratory conditions in sea or brackish water, about half of them become soft and rot in 3 or 4 months. In the field they are found abundantly among the beach débris and over the swamps for 2 or 3 months after their season of maximum ripening, but do not seem to survive much longer if not rooted and with rapidly growing tops. It is probable that *Avicennia* cannot stand as long an ocean voyage as *Rhizophora.*

Laguncularia seeds sprout more slowly than *Avicennia* and their seed coats stay intact until they take root and start growth. They float readily, because of their light pericarp, and seem to last longer in water than *Avicennia.* They probably float farther than *Avicennia* if they travel at the same speed.

Avicennia seeds and seedlings sink in greater numbers in the same water than do those of *Rhizophora* or *Laguncularia.* The seeds of the latter are smaller and harder and most likely to survive drying or possible transport by birds.

No significant tests or observations on the position of floating of either *Avicennia* or *Laguncularia* were made. The radicles and other parts of both these mangroves can survive long periods in salt water. The seeds of *Avicennia* are produced in great abundance, but those of *Laguncularia* are relatively scarce. In both cases most of the seeds fall in very shallow water or directly upon the swamp soils, so that few of them are carried into the open sea currents. None have been found recently washed ashore at the Tortugas. An experimental test of their dispersion should be conducted along the same route used for *Rhizophora* seedlings. The presence of both over a wide range indicates that they migrate efficiently, but probably not as efficiently as *Rhizophora* and related genera.

Currents and Other Factors as Dispersal Agents

Some observations and data on some of the currents effective in dispersal were included in the preceding parts of this section. The main littoral currents along the Florida coast are not, however, as important as the tide, local currents, flow of the estuaries, and winds in distributing the bulk of the seedlings. Most of the present mangrove areas in Florida, and probably throughout the world, have spread to cover nearly all the adjacent littoral that is habitable by mangroves. With the exception of the establishment of the plants on a few new islands or shoal areas at some distance from the seed-producing swamps, most of the effective dispersal is into adjacent swamp areas or onto adjacent shoals.

Strong ocean currents are not necessary, for tide and winds are most effective in such local dispersal. Very low tides may leave many seedlings stranded in favorable locations, and a very high spring tide may carry many of them far back into the swamps or marshes. Storm waves are instrumental in carrying some seedlings far inland beyond the range of the highest spring tides. Some of the river currents sweep many seedlings out into the shallows, whereas other rivers have so little current that they are paths along which mangroves may migrate inland with the flowing tide or wind currents. These local agents of dispersion are so affected by local conditions that no general statements can be made concerning them. Their vagaries may bring about very local distribution for a short time, causing many more seedlings to accumulate at some places than others.

The abundance of floating seaweeds and débris has some effect on dispersal, as the plants are aided or hindered by the nature and quantity of these materials. *Sargassum* and parts of the turtle and manatee grasses are the commonest débris found with the seedlings. On the mud flats or sandy shores this débris may accumulate and aid the anchoring and growth of the seedlings by holding moisture and adding to the organic materials in the soil.

Establishment

After migration there follows fixation or anchorage, growth, and finally reproduction; all are stages of establishment. Germination may precede, accompany, or follow migration, so that the term "ecesis" may be used in a qualified sense only. In many instances there is no migration of seedlings, for they take root and grow where they fall. In some instances they become temporarily anchored and may be washed out to become established elsewhere. Nowhere is there real dormancy of the seeds, so that germination is not one of the problems of establishment.

The establishment of the plants depends most upon the factors of tide, waves, submergence, and sedimentation. As previously noted, the position the *Rhizophora* seedlings take while floating aids in anchoring the plants in loose soil. The tide period and tidal range are important, for if the plants are only slightly stranded in loose soil at low tide they may be lifted out at high tide. If the tidal range is great, this action is more intense and the length of time and depth of submergence are greater. In such cases it is difficult for the plants to become fixed. Many plants are swept successively farther and farther into the interior of a swamp by tides and waves. In general, tides hinder establishment even if they do not prevent it. Strong wave action prevents establishment. Pioneer communities occur most abundantly where neither of these two factors is intense. In the swamps the effects of the waves are reduced, so that many more seedlings stay fixed than in areas outside the swamps.

Submergence to too great a depth, especially for a long period of time, will prevent growth or drown the plants. The prevailing height of the water above the soil in different zones of the swamps is probably a direct index of the height of water the seedlings will tolerate. Observations on young plants and recently anchored seedlings lead to the conclusions that: (1) young *Rhizophora* plants seldom become established in water averaging 2 feet deep; (2) young *Avicennia* and *Laguncularia* plants seldom withstand more than 6 inches average submergence. Constant submergence of *Rhizophora* seedlings to and a few inches above their tops does not seem to harm them, but constant submergence of the young stages of either of the other mangroves will prevent their growth. For these reasons, particularly in the case of *Avicennia,* the regularly flooded swamps do not have an abundant growth of young plants, especially over areas that are settling to lower levels and forming paludal basins. Thus a flooded scrub mangrove facies has few young black mangroves. White mangrove seeds, germinating more slowly than those of black mangroves, may float about until a low water level allows them contact with the soil. The fruit and seed parts of *Laguncularia* rot less slowly than the cotyledons of *Avicennia* when submerged.

Plantings were made to test the effects of depth and duration of submergence, and the results partly confirmed the above observations. Seedlings of all the three species were planted in rows from the highest to the lowest soils of the swamps. The results of these plantings at many localities and on three types of soil showed that: (1) the *Rhizophora* plants withstood constant submergence; (2) the *Avicennia* plants of over 2 inches top growth withstood nearly constant submergence, but the 4- or 6-leaved plants failed to develop if very frequently submerged; (3) the *Laguncularia* plants gave no consistent results, but most of them withstood some shallow submergence.

Since *Rhizophora* is the only species that can tolerate continued submergence for a year (as determined by keeping plants in tanks in the laboratory), it is best fitted to begin growth offshore and on shoals. Its ultimate permanent establishment depends mainly upon competition with the biota of these places, and the ability of the plants to stay anchored through periods of storm and wave.

Plantings of *Rhizophora* in similar soils but in deeper water than that in which they usually grow showed that they do not survive in large numbers a constant submergence depth of more than 6 inches above their tops. Of over 200 seedlings planted at station KL 6 in water averaging 6 inches above their tops, only 7 survived 9 months. A storm broke up the markers for this planting so that later observations could not be made. How some of the present mature swamps with soils almost constantly under 2 feet or more of water originated can only be surmised. There is no natural reproduction under the mature trees in such places, for the seedlings cannot establish themselves. Erosion, settling, or rise of the water level must have followed the primary growth in such a swamp.

Sedimentation about the roots of the young plants aids them in becoming established if the muds or sands do not cover and destroy them by too rapid accumulation. The latter is seldom the case. In general, marl sediments accumulate most rapidly and most satisfactorily about the mangrove seedlings.

Competition on the shoals or within a swamp is intense. Red mangroves, however, seem to find little difficulty in establishing themselves among the thickest growth of turtle grass and algae, for their vigorous roots are more than a match for these aquatics. Only storms and other disturbances check or destroy their gradual possession of the shoal, if the water level does not change too much. Within the swamps, the competition with other mangroves and associated plants results in the death of most of the seedlings. The survival of seedlings in sample plots of natural reproduction was about 20 per cent for *Lagun-*

cularia, 30 per cent for Avicennia, and 50 per cent for *Rhizophora* the first year. In bare areas of wet soils not constantly submerged, the crowded young plants nearly all survive the first year, but become thinned out with increase in size. In thick swamps only a very small percentage of any of the three mangroves grow to maturity.

The first-year rate of top growth is fairly rapid under normal conditions. *Rhizophora* produces 12 to 30 leaves and 6 to 24 inches top growth, *Avicennia* 4 to 14 leaves and 3 to 10 inches top growth, and *Laguncularia* 4 to 8 leaves and 2 to 8 inches top growth. The second-year and subsequent growth may be even more rapid, with branching, stilt root production in *Rhizophora,* and a rapid expansion of the root systems. After three or four years' growth the plants are so firmly rooted they are seldom dislodged.

Extreme exposure to winds and salt spray, and too much shade seem to retard growth or kill the plants. The type of soil and its depth also affect their growth, as on thin, heavy soils there is poor growth. Shifting soils are more injurious than dry ones and both are poor environments.

The ages of flowering and seed production have not been definitely determined. *Rhizophora* bushes as young as 5 years old flower. Many *Avicennia* bushes and some *Laguncularia* bushes less than 5 years old flower.

PLANTINGS

In the list of stations (pages 315–318) the locations of experimental plantings are given. These plantings were made to determine a number of points related to establishment and growth, such as the effects of competition, submergence, soils, and salinity, and also the gradual changes in the vegetation and soils at these places. This last objective will require a long period of observation. The oldest of the plantings are now only four years old, and most of them are too small to be significant in studies of vegetational and soil changes. On other points, some significant observations have been made and the results given above.

Three types of plantings were made: (1) small-area plantings along the margins and in different zones of the swamps, on all types of soil, and in different depths of water; (2) large plantings on shoals and coral-débris reefs; (3) plantings in the laboratory at Memphis, Tennessee, and the slat-house at Homestead, Florida.

The first of these plantings were rows of seedlings extending over typical portions of the swamps and out beyond the edge of the natural growth. Such plantings have shown that *Rhizophora, Avicennia,* and *Laguncularia* seedlings survive best on certain soils, in relation to certain water levels, and in respect to certain factors of competition. Most of these results have been given above.

The second set of plantings were in two places: one on three different marl shoals in Florida Bay and Barnes Sound; the other, the largest of all, on Long Key (pl. 12, fig. 1) at the Tortugas, where over 4000 *Rhizophora* seedlings have been set out in the calcareous sands and muds and on the coral ballast. All these large plantings are where mangroves are forming a pioneer community. The plantings were made to see if a thick stand of *Rhizophora* could be developed to hasten the natural processes of establishment of a swamp, and to make possible a record of mangrove growth and soil-accretion rate in such a dated swamp. The results so far are encouraging, as a large proportion of the plants have become well established (pl. 12, fig. 2) and it seems probable that many will survive to maturity. Of the 4100 *Rhizophora* seedlings planted at the Tortugas in 1938, about 3300 had survived to July 1939. The methods of planting can be improved; plantings in the Orient have become a common silvicultural practice according to Watson (1928).

The third set of plantings, in pots, tanks, and trays under artificial conditions, have been helpful in determining salt tolerance of the species, rate of growth, and reactions to a number of other conditions. Some of the *Rhizophora* seedlings have grown over 30 months and some of the *Avicennia* and *Laguncularia* seedlings over 24 months in salt-free water. After 24 months the differences in growth of *Rhizophora* in salt or fresh water and in marl or peat soil seem to be negligible, with but slightly better growth in peat than in marl soil, and taller plants with more leaves in fresh than in salt water.

PART II. THE GEOLOGIC RÔLE OF MANGROVES

INTRODUCTION

Some preliminary observations as to the geologic rôle of mangrove vegetation are worth noting here. The apparent vegetational succession of the mangrove communities and the nature of the soil profiles are indicative of appreciable accretions of soil. Comparisons of old and recent maps, and of some old maps with recent photographs, show sizable changes in land area, most of which are increases. It is easy to observe that water washes in marine sediments and that organic matter accumulates in the swamps in sufficient quantities to raise the level of the soil and in many cases to extend the coast and form islands above high-tide level, though it is difficult to determine the rate of accretion and the total land-area changes for any one region.

It is probable, moreover, that mangrove soils may also be used to interpret past geologic changes. From detailed studies of soils of salt marshes much has been learned by Chapman (1938), Johnson and York (1915), Nichols (1920), C. A. Davis (1911), Nielson (1935), Yapp, Johns, and Jones (1917), and many others concerning the nature and rate of accumulation of silt, muck, and peat deposits of coastal areas of New England, England, and the continent of Europe. The coastal geology and accretion processes of salt marshes and mangrove swamps are so similar that what has been learned of the former may be to some extent applied to the latter. The interpretation of deep cumulose deposits in relation to coastal stability and instability is most pertinent. The maximum tidal range limits the depth of marsh and mangrove deposits. If there is coastal stability over a long period of time, the plant zonation is relatively narrow or absent and soil deposition above the level of the highest marshes or mangrove swamps ceases. When subsidence or emergence, or an actual rise or fall of the sea level, occurs, the marine deposits respond according to a characteristic pattern. Thus their depth and nature may be used as an index of the changes of the tidal plane.

C. A. Davis (1911) states that "the rate of subsidence is equal to the rate of accretion minus the rate of rise of the marsh level in relation to the tidal plane." Thus, if there are remains of plants in the peat outside and seaward of their present zones, the rate of accretion has been less than the rate of subsidence. If there has been appreciable emergence, or lowering of the sea level, salt-water peat will be found inland and above the present sea level. Using these and other principles, Chapman (1938) and Johnson (1913) have calculated the age of marshes of the different periods at which the coast was stable, submerging, or emerging.

Such relatively accurate methods have not been employed in mangrove regions and probably would involve more difficulties. It seems reasonably certain, however, that mangroves not only build up marine sediments and cumulose soils within the limits of the present tidal range, but also have been building soils along the coasts for a long period of time as the sea levels changed.

As we have seen in part I, many features of the plant communities and factors of the environment are related to the geologic rôle of mangroves and should be kept in mind. In the "History and Introduction" some of the literature that stressed this geologic rôle was reviewed. The importance of mangroves as agents in soil-accretion processes has been recognized for a long time, but few of the observations about mangrove vegetation have included any thorough study of the processes of sedimentation and accumulation of soil materials. Some of the early and recent investigators who consider mangroves important geologic agents base their conclusions upon evidence that is too superficial. Other investigators consider mangroves of little importance in sedimentation processes. Typical of the latter is the study by Freyberg (1930) of part of the Brazilian coast. He states: "Die Mangrove ist folglich kein Landbildner; sie kann höchstens die letzte Phase der Landbildung beschleunigen und braucht also Räume mit positivem Sedimentgefälle." He recognizes, however, that the mangrove plants contribute to the accretion processes, from the pioneer stage of *Spartina brasiliensis* on the offshore mud flats to the tall, close stands of red mangroves of the swamps, and he probably would have attributed to mangroves an even more important rôle had he studied the soil profiles of the swamps.

The information about mangroves and soil accretions is scanty and inconclusive, largely because no long-term study has been made. The problem of the geologic rôle of mangroves is not a simple one. Many evidences of land-area increases have been found, and some of the soil profiles indicate the major features of the accretion processes. The marine and biotic factors that promote accretions are fairly definite, but the geology and possible coastal changes of this part of Florida are not very clear.

The following account of the evidences of land-building, and interpretation of the factors involved, is not by any means complete. Future reports may include some additions and alterations. In spite of this inconclusiveness, the study affords reliable bases for the conclusion that new land areas are being built up in Florida.

EVIDENCES OF INCREASES IN LAND AREA

Some good evidences of land-area changes were obtained in Florida through a fortunate set of circumstances. Recent land and aerial surveys have been made of many of the mangrove areas along the southern coast of the state, on the basis of which new charts and maps have just been published or are being prepared by the U. S. Coast and Geodetic Survey. Some excellent aerial photographs made by the U. S. Army Air Corps in its Fourth Photo Section Survey were available for study and could be used to great advantage with the new land surveys. Old maps and charts were compared with recent ones and the land changes located. Some of the maps and charts are drawn in such detail, and the photographs are so large, that small changes could be noted. By means of these comparisons of old and recent information the best areas of change were selected

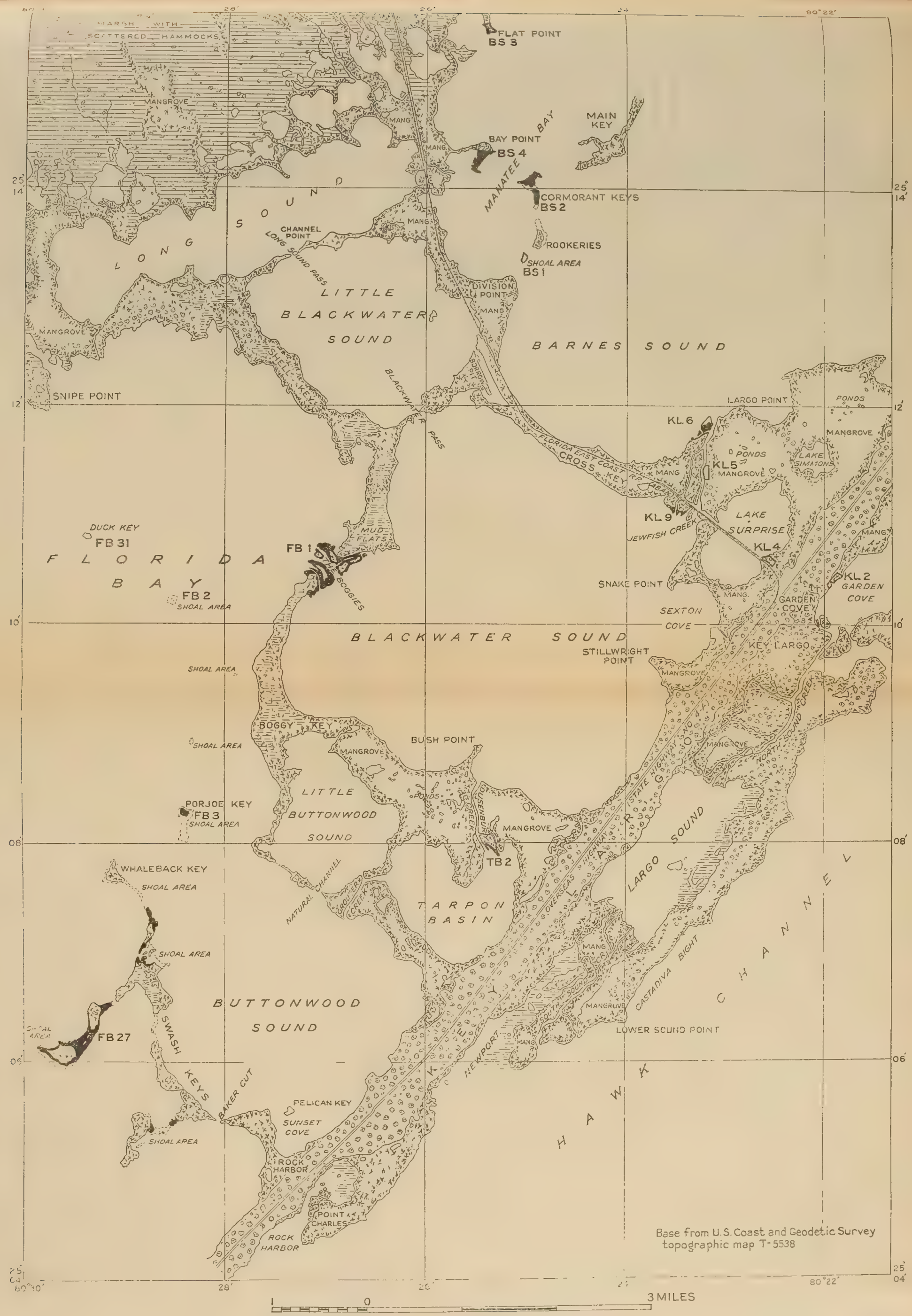

FIG. 6. Part of U. S. Coast and Geodetic Survey topographic map no. T-5538, showing location of stations, and some recently developed swamps and land (shown in solid black). Source of the data for the map from land surveys and air photos.

and many of the localities visited. After numerous such visits, and elaborate checking of the map data with field observations, it is now possible to state that there are many instances of appreciable land increases in and about the shoal waters of southern Florida (region B, fig. 1). Some of these changes are shown in figures 6 and 7, but not nearly all of them can be included here. For convenience the recent charts and maps have been listed below (pages 386–387). Some of the older charts and maps bear the same numbers as these recent ones and can also be obtained from the U. S. Coast and Geodetic Survey. Some of the oldest charts are not readily available. The dates of the authorities are given on these maps and charts, but not all the information about the dates of the surveys can be obtained from them.

In addition to the above sources, the study by Millspaugh (1907) of the Sand Keys was used in comparison with recent maps to locate changes among those islands, which extend from Key West to the Tortugas inclusive. In other regions of Florida information was less accurate. There are some aerial photographs of the region from the Ten Thousand Islands to Cape Sable which when compared with the maps indicate land changes, but this whole area has never been adequately surveyed and the comparisons do not give accurate results.

Even though map comparisons cannot be made along all the coast, the situation of the coastal bars, shoals, and islands is often indicative of definite changes due to large-scale accretions of sedimentary and cumulose soils. This is particularly true of the island-studded region between Cape Sable and Cape Romano. Here the air photographs show that most of the Ten Thousand Islands are little elongated mangrove islands stretched out end to end in nearly parallel rows, all trending southeasterly, parallel to the general direction of the coast line. They are nearly all little shell bars on which mangroves have grown and in many instances aided the accumulation of sands and muds to form uninundated soils above high-tide level. Most of these islands are larger at their southeast ends, indicating that a uniform current from that direction aided in their formation. Soil studies showed that some of these islands are enlarging or building up to higher levels at a fairly rapid rate, although there is little map evidence to confirm this observation.

In the same region an interpretive study of the present dissected land masses of the coast suggests that they may have formed from the coalescence of many small islands. The numerous tidal rivers that drain the mangrove swamps and Everglades are nearly all connected by channels, bays, and basins. The rivers may be the nearly closed remains of tidal passes which have kept their courses scoured out as the islands joined together to form large blocks of swamp land, marsh, and some hammock land. Some of the cross-connections are shallow bays, such as those from Alligator Bay to Sunday Bay, which may have been larger and similar to the present Chokoloskee Bay. The present Ten Thousand Islands may similarly coalesce in the future.

The map comparisons were supplemented by field observations and by the reasonably reliable observations of persons living or working in these regions many years. For example, Mr. A. R. Livingston, of Homestead, recalled many instances of land-area changes, and some of decided increase in the soil level. Landowners furnished valuable information, of which that given by Mr. McRae, of the Key Farm Land Company, is typical. He has surveys made in 1873 to 1875 of some property on Key Largo and adjacent islands. Near Steamboat Creek, in or near sections 16, 21, 22, 27, 28, township 59, range 40, his property has increased over 15 acres. In a section of the Anglefish region of township 58, range 41, a 50-acre tract has increased to 60 acres. Similarly at Palo Alto Key, near Broad Creek, deeds of 1873 refer to 147 acres, while the property is now 180 acres. Riparian rights are considered important by all landowners of mangrove regions, as the land increases may be appreciable.

In numerous instances fishermen and guides could point out changes they had observed in the past 20 to 40 years. Some of their observations were checked by means of the map comparisons, and in every case they were found reliable. It was especially interesting to visit a swamp which a guide had seen develop in 30 years from an open shoal into a thick swamp forest. In many instances the growth rings of *Rhizophora* corresponded to the number of years the guide gave as the age of the swamp.

It is difficult to estimate the number of acres involved in these land increases. Many of the regions are so nearly flat that an inaccuracy of a few inches in estimating tidal coverage affects large areas. The charts and maps do not satisfactorily show the mean high-tide level. If all areas above mean low tide are land, the areas of increase are very great, but if only those definitely above mean high tide are considered land, then the areas of increase are small. Field observations show that many places indicated on the charts and maps as land are usually covered by all but the lowest tides. It is best, however, to consider any well-developed swamps as land, not sea, for there seems ample evidence in many instances that the submerged parts will eventually be above any of the normal high tides.

Calculations of land areas are also affected by temporary changes in soil level which occur without any extension of the land, owing to tide-level changes during the seasons and to marine factors of several kinds that cause sediments of the shoals to shift about. It is probable that about 1500 acres of new mangrove swamps have appeared in region B (fig. 1) within the past 30 or 40 years, especially if mature mangrove swamps are included as land, but the qualified use of the term "land" must be kept in mind.

List of Charts and Maps of the U. S. Coast and Geodetic Survey with Reference to the Regions of Mangrove Study Shown in Figure 1

Region A:
Nautical charts nos. 1245, 1246.
Region B:
Nautical charts nos. 1249, 1250, 1251.
Topographic maps nos. T-4577, T-5439, T-5440, T-5441, T-5442, T-5538, T-5539, T-5540, T-5541, T-5542, T-5543, T-5544, T-5545, T-5546, T-5547.

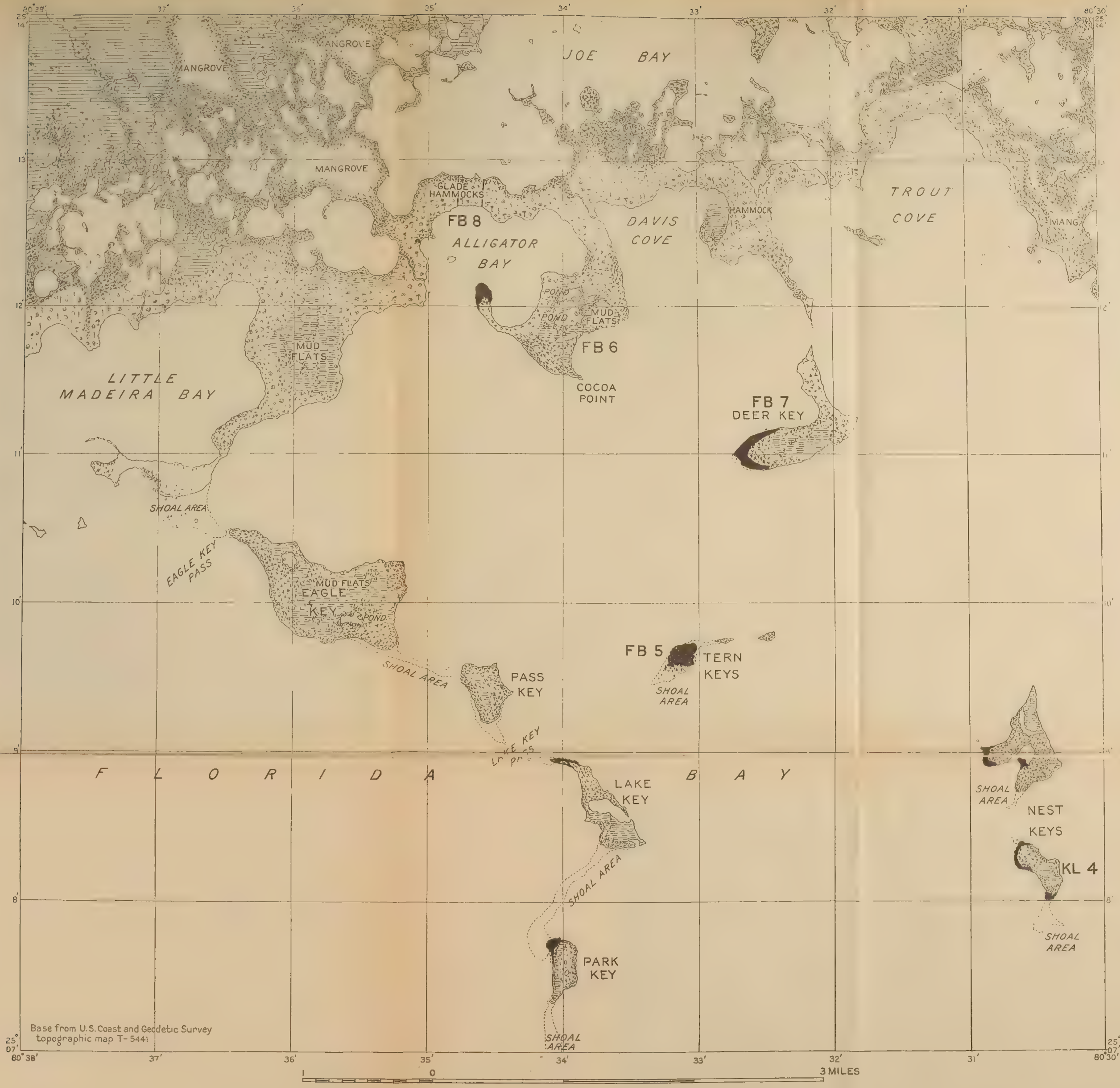

Fig. 7. Part of U. S. Coast and Geodetic Survey topographic map no. T-5441, showing location of stations, and some recently developed swamps and land (shown in solid black). Source of the data for the map from land surveys and air photos.

Region C:
Nautical charts nos. 1253, 1254.
Topographic maps nos. T-4457, T-4458, T-4459, T-4460, T-4461.
Region D:
Nautical charts nos. 1256, 1257, 474, 586, 178, 179.
Region E:
Nautical charts nos. 1252, 585.

THE SOIL PROFILES AND THEIR SIGNIFICANCE

Although, as described in the section on "Soils and Substrata" above, great care was used in obtaining samples from different levels, it was almost impossible to do so without some compacting and mixing of the soils. In many instances it was difficult to clean the instruments properly between samples. The field conditions hindered the work, as in some places the swamp soils were so loose or slippery that a good footing could not be obtained for the heavy work required in forcing the sampler through the soil or drawing it back with a load. The flooded condition of most parts of the swamps added to the difficulty of getting up samples without pollution. In spite of these difficulties most of the samples were distinct enough for good observations of texture, structure, and gross composition of the soils at the different levels or in different zones. The greatest difficulty was in determining the exact levels of different strata in some of the swamps. However, in most cases these levels were significant without determination to within an inch or a few inches.

The soil profiles have been classified according to their general composition, whether homogeneous, heterogeneous, or layered. They might also be classified on the basis of the type of vegetation now covering the soil, or the probable type of vegetation that formerly covered the soil and contributed most to the cumulose soil materials. It might seem that these two methods could be combined, but this is not the case. The same type of mangrove community does not occur regularly on any one type of soil as regards its homogeneity or condition of layering. Moreover, the same type of soil as regards its origin from plant and animal remains does not occur consistently with a certain type of vegetation. Therefore, the soil profiles presented in table 14 are grouped according to homogeneity or condition of layering of the soil, and the vegetational covering is indicated. These are only a few of the more than a hundred profiles studied, but are selected as representative of nearly all conditions. Some composite profiles of soils of adjacent communities are given to show more clearly than a single profile the successional relations of some of the plant communities.

The gross features of the texture, arrangement of parts, color, friability, and wetness or dryness, as well as the whole and fragmented remains of organisms, were studied in most instances. Some microscopic studies were also made. The minute structures of certain plant parts, such as the idioblasts of macerated or decomposed leaves, tannin cells, the pithy cortex of roots, and corky and endodermis tissues, could be used to identify the plants from which some of the peat and muck soils were formed. From these two types of study, it was possible to assign a definite origin to many of the peat and muck soils. Thus a vegetational history can be obtained for many of the soil profiles.

Less detailed studies were made of the sedimentary marine soils than of the cumulose organic soils, but these may also indicate their mode of origin, although an interpretation of the profiles is more difficult. Some studies were made of the contributing marine organisms, mostly shell animals, of the marl and calcareous sand soils.

Many other features of the soils have been discussed in the section on "Soils and Substrata" above. It is well to recall that the basic soils are siliceous sands, calcareous sands, marl muds, and mixtures of these three. The soils of high organic content are mostly cumulose on top of these basic soils, but some plastic peat and muck soils also occur that are sedimentary and in some instances transported into the swamps. Most of the deep peat soils have formed *in situ* and represent the condition of the vegetation over a long period of time. Mixtures of marine sediments and organic soils of plant remains are common and very difficult to interpret. In some instances the soils are well layered, with alternating strata of sedimentary and cumulose soils, or of different types of either one of these two. The significant thing is that the profiles of some of these swamp soils can be used to interpret the processes that formed them, particularly the changes in vegetational covering and the changes in soil level, or both. In general the term "halotropic" can be applied to many of the mangrove soils, for they show changes with succession of the plant communities, and the soils were developed in salt water.

Many of the profiles are indicative of progressive soil accretions with gradual raising of the soil level, and this feature of the profiles is most indicative of the land-building processes (Stokes and Roberts, 1934). The layered or partly stratified soils are most indicative of progressive accretions, and the heterogeneous soils least. Some conditions difficult to interpret should be noted. When a homogeneous deep profile is of nearly pure fibrous peat of *Rhizophora mangle* origin, and is covered with *Avicennia* and salt-marsh plants, the profile is indicative of a recent change in the vegetation. The difficulty in this instance is in explaining how a deep layer of peat could occur without a definite rise in the soil level and a consequent change in the vegetation. This situation may be due, as pointed out below in the section on "Geology and Coastal Changes," to a gradual rise in the sea level accompanying the growth of the swamps. In some cases the layers show that the normal sequence of vegetation did not occur, since a soil associated with plants of higher-level communities is below soils associated with plants of lower levels. Some oscillation due to settling of the soils, erosion, or other change is indicated.

In the case of many of the nearly pure marl soils, in some of the siliceous sand soils, and in nearly all the pure calcareous sand soils there is little or no indication of the plants that contributed to their accumulation. Remains of plants are often so scarce and the parts are so scattered or of such heterogeneous origin that they cannot be identified. Similarly, plastic peat soils indicate little of their origin, since the plant parts are so finely divided and so mixed. As in the case of the sand and marl mud soils, the materials are transported from their point of origin. However, the levels of these soils change, as indicated by layering, cross-bedding, and interbedding with soils of higher organic content or with each other. Thus even though their vegetational or other organic history cannot be determined accurately, the profiles show in many instances that marl soils and sand soils are fairly definitely deposited at higher and higher levels in many of the swamps.

Five homogeneous soil profiles are given in table 14 that show some of the conditions of peat, marl, and sand soils in both Rhizophora and Avicennia communities. Deep peat soils are indicative of deposition of plant materials over a long period of time without much change in most conditions within or near the swamps. This is particularly true of profile 45, in the mature mixed mangrove forest along the shores of Shark River. In contrast with deep peat soils, deep marl soils do not indicate any long-term deposition within mangrove swamps. The marl deposits are most often of marine origin, being caused by slack water and shifting currents in the shoal waters. The situation at station BS 2 (profile 15) is typical of such conditions. A vigorous mature Rhizophora consocies has resulted from a 30-year growth on a nearly emerged shoal that was composed of marl mud and calcareous ooze. The red mangroves have caused the addition of some marl to the top soil of the shoal, but have not added enough plant débris to change the character of the 6-foot-deep marl. Many similar situations are found in the shallow-water basins of southern Florida. They indicate only the first, or nonpermanent, stages of mangrove swamps, but in general such marl soils are of some significance in a consideration of coastal changes. However, it is only in cases where peat deposits overlie marl (profile 88) or are interbedded with it that the profiles indicate definite changes in sea level.

Profile 51, in a silicon sand region, is a shallow layer of fibrous peat mixed with sand over the deep primary sand of the coast. The swamp is mostly of *Avicennia* and not flooded at low tide. The depth of the peat and the level of the soil indicate that the swamp has added débris on top of the sand for a fairly long time. Young swamps on sand soils have little or no upper peat layer, the sand being looser and grayer near the surface than below (profile 62). The few studies of deep calcareous sand soils (profile 75) showed that no definite fibrous peat layer was developing, but no soils of old mangrove communities on these soils were studied.

Heterogeneous soils are usually found at localities of less uniform conditions of sedimentation and organic deposition than those of homogeneous soils. Thus at station BB 1 (profile 1) the soil is mixed marl and peat, forming a muck with little or no layering of organic and sedimentary materials. This coast is now being eroded and marl is being washed in over the swamp. The probable history of this coast is of periods of relatively quiet waters, little erosion or deposition of marl, and some peat deposition, alternating at relatively short intervals with more agitated waters which cause more marl to be washed into the swamps.

Under such circumstances the plastic marl mixes with the peat and no definite layers are formed. Profile 57 indicates much the same condition, but is from a silicon sand region. The fibrous peat at this locality is mixed with sand that is nearly constantly washed into the swamps. Such heterogeneous soils are almost as indicative of gradual accretions as are the homogeneous soils, but it is more difficult to estimate the level or levels at which the different accretions began.

The layered soil profiles indicate best of all the mode of soil accretions in mangrove swamps. One of the best examples is profile 6, where stratification extends down 11 feet to the rock bottom. The heavy gray surface marl is from 12 to 18 inches thick and above the usual high tide. In and below this layer the root zone of the present plants extends to about 4 feet through mixed dark marl and some fibrous materials, mostly formed of the living roots. From 4 feet to about 8 feet there is a distinct layer of fibrous peat, mostly of red mangrove origin, and below this is a 1- to 2-foot layer of marl with a 1- to 2-foot layer of marly plastic peat between it and the basal rock. Thus the profile exhibits five fairly distinct layers, which may have formed thus: (1) the top heavy marl layer was recently washed over the swamp by high water; (2) the root zone was formed recently from water-borne sediments and plant remains; (3) the deep, 4-foot layer of fibrous peat was formed at a time when the swamp surface was at a lower level than now and little marl was washed in or remained with the developing peat in a vigorous mature Rhizophora consocies; (4) the lowest marl layer was probably the original marl of the shoal on which the mangroves began growth; (5) the plastic organic layer at the bottom is probably due to the downward infiltration and settling of fine materials which collected just above the rock.

Other layered profiles are similar though some are less deeply layered, as in profile 27 at Murray Key, which has a 2-foot layer of peat under an 18-inch layer of recent marl, and over a 5-foot layer of firm marl that probably represents the shoal upon which the red mangroves began. The top marl layer is seldom flooded by the tides and has been recently washed in over the peat. The island is located in Florida Bay, where many similar profiles are found.

Three conditions of development of soils exist in Florida Bay: (1) incipient islands on submerged shoals with no peat layer; (2) mature middle-age islands of rich mangrove growth, mostly mature Rhizophora consocies, which are forming beds of peat below the tide level; (3) older islands with fringes of *Rhizophora* and interiors of Avicennia salt-marsh associes which are being surfaced with marl above or near the present tide level. Low Key (profile 88) is an example of the stage between condition 2 and 3, as it has developed a deep peat layer over the basic marl and the surface has not yet been covered by marl. Murray Key is an example of condition 3 of the old islands, and profile 15 from station BS 2 in Barnes Sound is an example of the incipient island stage.

Many more profiles could be cited or described to show how there is a more or less definite sequence of building up of insular and coastal soils from submerged marl or sand shoals. In general, the vegetational succession from the pioneer Rhizophora family to the Conocarpus transition associes (see "The Mangrove Communities") is the result of the successively higher levels to which the soil is built as the swamps grow older. Unfortunately these different levels are not all distinctly stratified, layering depending a great deal upon the character of the sedimentary soils in the shallow waters near the swamps, and the best layering of soils occurs only in the marl soil regions where the fine marl is easily washed over the peat after the latter has formed.

The profiles of the inner zones of the swamps were more difficult to obtain, but some of them provided a good series of soils from the pioneer stage to the definitely upland forest or inland marshes. Profile 73, of the Florida Bay–Cocoa Point region, is from the Conocarpus transition associes zone and has two upper layers of marl, with some shell, over a peat layer which lies over the basic marl of Florida Bay. The uppermost marl layer is rich in humus and granular, and it supports the buttonwoods and some tropical hammock plants. This layer is not easily distinguishable from the heavy marl layer below it, but the top layer is above the present normal high-tide level. The peat layer is below the tide level and was laid down under water by the early growth of the swamp. The upper marl layer was washed inland by high water, over the wet marl layer that occurs at the tide level, and is now an upland soil with humus and supports nonhalophytic plants.

Another layered profile indicative of the mangrove succession is profile 22, in an incipient hammock along the tension zone between mangrove swamps and the Everglades marshes. Here the upper leaf-mold layer is underlain by red mangrove peat over the shallow marl that covers the rock of this part of the Everglades. The whole profile is less than 4 feet deep but indicates how hammock plants can begin growth directly on top of mangrove peat.

From these and many other profile studies it seems reasonably certain that: (1) the layered condition of the soils occurs mainly where marl and peat are associated; (2) in general, marl underlies and overlies peat; (3) the lower marl is of marine shoals and bars, and the upper marl is washed in at about the tide level; (4) the thickness of the peat is indicative of the length of time the swamp was submerged and had a vigorous growth of mangroves, mostly *Rhizophora;* (5) a layer of peat much deeper than the present tidal range suggests a gradual increase in the sea level, as discussed below (see "Geology and Coastal Changes").

From a series of profiles taken at the same station in different zones of the swamps, a similar indication of progressive changes can be obtained. Two such series are given in table 14 under "Composite profiles." The profiles from station FB 6 will illustrate the conditions. Profile 71, where the young red mangroves are beginning on a spit extending into Mud Bay, is the homogeneous marl mud of the bay 5 feet deep to the rock bottom. Profile 72, taken at the inner edge of a mature Rhizophora consocies, shows a 2-foot layer of mangrove peat above the basic marl. Some marl is being washed in over the inner part of this zone. The character of profile 73, in the Conocarpus zone, has been described. Inland

TABLE 14. *Representative soil profiles in mangrove and associated communities*

Profile No. *	Station	Description
		HOMOGENEOUS TYPE
1......	BB 1	Mature Rhizophora consocies. 7 ft. nearly pure raw fibrous peat, over coral rock. Mostly of *Rhizophora* origin. Top to about 5 ft., firm fibrous peat with darker organic sediments increasing downward, lower 2 ft. nearly plastic peat. Interlacing network of roots and rootlets down to 6 ft. Peat and rootlets reddish brown. Peat very friable when dry.
3......	KL 3	Flooded Avicennia associes. 12 ft. nearly pure fibrous peat, over thin layer of marl and basic rock. Top 3 ft. firm fibrous peat with many *Avicennia* roots, but most of peat of *Rhizophora* origin. Less fibrous below with marl materials increasing. From 10 ft. to bottom, muck mixture of plastic peat and marl.
15......	BS 2	20-year-old mature Rhizophora consocies on marl mud shoal. Soft marl mud ooze near surface, becoming firmer marl below to 6 ft., over rock. Fragmented remains of marine animals, some calcareous algae, calcium carbonate granules. Some aquatic and mangrove plant material in upper ooze layer.
45......	SR 2	Old mature mangrove forest associes. 12 to 14 ft. mixed-type fibrous peat, over rock. In some places 6+ in. marl mud covers surface, and marl and shell materials are mixed with peat. Top to about 5 ft., reddish-brown raw fibrous peat with marl, shell, and sand materials increasing downward; below 10 ft., over 40 per cent of soil is of these materials. Peat becomes darker below and less fibrous. Roots of standing trees penetrate freely to about 9 ft. Peat of mixed *Rhizophora* and *Avicennia* origin, mostly the former.
51......	LB 3	Avicennia salt-marsh associes on siliceous sand. 2 ft. fibrous peat, over coarse siliceous sand. Peat of *Avicennia* and *Rhizophora* origin.
62......	SB 9	Young pioneer Rhizophora family on siliceous sand. Top to about 3 ft., coarse gray sand, becoming yellowish below. Roots penetrate sand to about 3 ft.
75......	BK 2	Laguncularia consocies with a few *Rhizophora* around pond. Coarse calcareous sand and coral fragments. Top to about 2 ft., brownish sand impregnated with organic materials, becoming nearly white below.
88......	FB 20	Avicennia salt-marsh associes with outer fringe a Rhizophora consocies. 4 ft. fibrous peat with some marl and shell fragments, over about 5 ft. basic marl, over rock. Peat of *Rhizophora* and *Avicennia* origin, top layer most fibrous, becoming plastic below.
		HETEROGENEOUS TYPE
1......	BB 1	Mixed mangrove fringe along eroding shore. Mixed peat and marl not distinctly layered. Top few inches nearly pure marl, over 5 ft. peat and marl, forming muck, over rock. Plastic peat and marl at lower levels, fibrous peat and shell fragments at upper levels.

*The numbers are those assigned the samples in the field, and have no relation to their significance.

(*Continued on following page*)

TABLE 14—*Continued*

Profile No. *	Station	Description
		HETEROGENEOUS TYPE—*Continued*
57......	SB 1	Avicennia salt-marsh associes on mixed fibrous peat and coarse siliceous sand. Top to 1 ft., loose fibrous peat and sand. 1 to 3 ft., nearly pure fibrous peat, with sand increasing below and becoming lighter in color; pure siliceous sand at 5 ft.
83......	EC 2	River fringe of *Avicennia* and *Rhizophora* on mixed fibrous peat and sandy muck. Sand siliceous. Peat fibrous to about 3 ft., with sand increasing below; siliceous sand at 5 ft.
		LAYERED TYPE
6......	KL 3	Avicennia salt-marsh associes with some *Rhizophora* along outer edge. Top to 1 ft., gray heavy marl with roots of salt-marsh plants and some mangroves; 1 to 2 ft., darker marl with many *Avicennia* roots; 2 to 4 ft., nearly pure heavy gray marl; 4 to 8 ft., nearly pure fibrous peat mostly of *Rhizophora* origin; 8 to 9 ft., marl with some plastic peat; 9 to 11 ft., plastic peat and marl. Surface marl from 1 ft. to 18 in. is above usual water table.
22......	CS 1	Young *Paurotis* palm and hardwood hammock. 9 to 18 in. leaf mold, over 6 to 12 in. *Rhizophora* raw fibrous peat, over 6 to 9 in. marl, over rock of Everglades.
27......	CS 8	Old Avicennia salt-marsh associes on Murray Key. 18 in. gray, heavy, but partly granular marl, over 2 ft. dark reddish-brown fibrous peat, over 5 to 6 ft. basic marl.
73......	FB 6	Conocarpus transition associes with some stunted tropical hammock plants. About 16 in. marl containing some humus and granular, over about 1 ft. heavy marl with shell fragments, over fibrous peat to about 5 ft., over basic marl. Peat mostly of *Rhizophora* origin and mixed with marl.
27......	SB 1	Avicennia salt-marsh associes. 3 ft. peat with some siliceous sand, over 2 ft. gray sand, over yellowish sand.
		COMPOSITE PROFILES
71......	FB 6	Pioneer Rhizophora family on extending spit. 1 ft. loose marl mud ooze, over 5 ft. firmer marl, over basic rock.
72......	FB 6	Inner margin of mature Rhizophora consocies. 2 ft. fibrous *Rhizophora* peat, over 5 ft. marl.
73......	FB 6	Conocarpus transition associes. About 16 in. granular marl rich in humus, over about 1 ft. heavy marl, over about 3 ft. peat, over basic marl. Upper marl layer above high-tide level. Both upper marl layers with shell.
48......	CR 3	Mixed mangrove forest associes with *Rhizophora* dominant. 9 ft. fibrous peat, becoming more plastic, and with more shell and sand in lower part.
49......	CR 7	Mixed Avicennia and Conocarpus associes near edge of Everglades marsh. 4 ft. mixed peat, shell, marl, and sand. Peat material of both mangrove and marsh plants.
50......	CR 9	Everglades marsh with colonies of palms and *Conocarpus*. 2 ft. peaty muck. Fibrous materials mostly of marsh plants.

*The numbers are those assigned the samples in the field, and have no relation to their significance.

from the Conocarpus transition associes a thicker growth of hammock plants occurs, but no profile was obtained; here the top layer of marl is even more granular, darker, and of higher elevation than at the location of profile 73.

FACTORS THAT PROMOTE SOIL ACCRETIONS

Introduction

The factors most influential in promoting soil accretions are: (1) molar, (2) physicochemical, (3) biotic. The relative importance of each of these depends upon the situation and the type of vegetation. The molar factors are mainly marine currents and are most directly the cause of sedimentation. The biotic factors are mainly vegetational and are most important during the later stages of soil accretion in the swamps. The physicochemical factors are of little real importance but are of some interest.

The first stages of accretion consist in marine and estuarial sedimentation. The formation of shoals, bars, and flats in the shallow waters where mangroves begin is due mainly to molar agencies. These include the littoral currents, tides, and winds that shift the materials about, cause turbidity, fragment rock and animal remains, and allow the settling of particles. To a less extent the physicochemical precipitation of carbonate, muds, etc. contributes to the basic soils or substrata upon which the swamps begin. Locally, mollusks, corals, calcareous algae, and other marine organisms are of importance. These sets of factors normally operate together, with local differences in the effectiveness of any one. The soils that form on top of the primary deposits have likewise accrued through the action of these same factors, but in general the biotic factors become more important with increasing age of the vegetation, causing the development of the upper layers of organic cumulose soils. In the swamps and marshes the frequent baths of detritus-laden water driven in by onshore winds and the tides, or brought from the interior by floods, cause the accumulations to pile up around the mangrove roots and marsh plants. The unusually rapid rate of these accumulations among the mangrove roots is the significant feature of their rôle in promoting soil accretion.

Molar Factors

The prevailing near-shore currents, tides, and winds along the Florida coast bring about conditions very favorable to sedimentation. This is particularly true in the whole south Florida region between the Gulf Stream and the mainland. The islands and shallow basins here have been thoroughly studied by a number of persons, and the deposits analyzed as to composition and mode of origin. In his study of the shallow-water marine deposits along the Florida Reef Tract, Thorp (1935) shows how the molar agencies carry, sort out, and cause the deposition of calcareous and other materials, and Vaughan (1935) gives significant data for currents in this region. A southward- to westward-flowing countercurrent carries materials through the shallow waters from the Atlantic coast along the Florida Keys out as far as the Tortugas, affecting regions B and E (fig. 1). The current is enhanced by the prevailing easterly winds, and with surface wave action is often strong enough to cause noticeable turbidity of the water. The tidal currents in general run north and south across the main east-west axis of the southern portion of this countercurrent (fig. 5). The curve of the Florida Keys parallels the path of the countercurrent. Vaughan (1935) secured many data on velocity and direction of currents under different conditions of tide and wind. He gives measurements taken at three places in Hawk Channel, where the countercurrent flows, and two sets of data taken north and south of Knight Key, that show the tide currents. The averages of his results are given in table 15.

TABLE 15. *Current averages, Florida Reef Tract*
(After Vaughan, 1935)

Location	Tide	Velocity (knots)	Direction
Caesar's Creek Point....................	Flowing	0.180	N. 60° E.
Caesar's Creek, mouth..................	Flowing	0.310	N. 60° E.
Caesar's Creek, mouth..................	Ebbing	0.454	N. 85° E.
Rodriguez Key..........................	Flowing	0.224	N. 50° E.
Rodriguez Key..........................	Ebbing	0.194	S. 5° W.
Knight Key, north side..................	Flowing	0.929	S. 15° E.
Knight Key, north side..................	Ebbing	1.391	N. 40° E.
Knight Key, south side..................	Flowing	1.013	S. 5° E.
Knight Key, south side..................	Ebbing	1.278	N. 7° W.

The data for three places in the Hawk Channel show slow movement of the current from east toward west, but are not conclusive regarding the countercurrent. Better data on this countercurrent and cross-currents are obtained from pilot charts of the U. S. Hydrographic Office, and the current tables of the U. S. Coast and Geodetic Survey give more information on the strength and direction of cross tidal currents between the Florida Keys. Five stations from Miami to Boca Grande Channel, near the Marquesas Keys, are given in the Atlantic Coast Current Tables, and the data indicate the same condition of cross-currents that Vaughan recorded at Knight Key. The direction of the flowing tide at three of these stations near mangrove areas is between S. 25° W. and S. 45° E., with an average velocity of 1.3 knots. These figures indicate a strong tide current across the more gently flowing countercurrent. The Gulf Stream that sets eastward and northward through the Strait of Florida has a mean velocity of about 3 knots 10 or more miles out from the Florida Reef Tract and does not affect the currents near shore. Some of the currents and the location of stations where current observations were made are indicated in figure 5.

The countercurrent moves materials southward and then westward to aid in the extension of the chain of islands out to the Tortugas and in the continual renewal of materials along the whole of the Reef Tract. The cross-tides and winds tend to wash materials from the reefs and flats into the basins behind the keys, and in general to dam up and impound water in them, causing the prevalent condition of slack water in the shallow basins. Few strong currents exist in them. Winds may churn up the water, agitate sediments, cause temporary wind currents, and pile up materials near islands or over shoals and bars. Drainage from the islands and mainland adds other currents and sediments. The result is the creation of all sorts of vagaries of currents, with sorting and re-sorting of sediments, and with deposition and erosion taking place at irregular intervals and different places. In short, favorable conditions for sedimentation of coarse to fine materials exist along the whole of the chain of islands and especially in the shallow-water basins behind them. Sometimes islands or shoals are obliterated, or appear for indefinite periods.

The U. S. Coast and Geodetic Survey hydrographic and nautical charts of the basins in Florida Bay, charts 1250 and 1251, and topographic maps T-5538 and T-5441 show how the islands and shoals are arranged more or less around these basins within the bay, indicating that there are eddies around whose borders deposition takes place. An elevation of a few feet in the floor of this bay would produce a number of ponds with narrow channels between them. The mangrove islands around such basins are the highest soils of this complex of shoals and bars. Their shape and direction of growth indicate past and present conditions of sedimentation. In the Deer Key to Tern Keys part of Florida Bay (fig. 7) the islands are building westward, in other sections they are extending southward, or even eastward. The present shoals indicate the probable shape and direction of future islands.

An interesting illustration of the probable effect of currents in creating conditions favorable for mangrove islands is furnished by the observations of some persons as to the effects of building the Florida East Coast Railroad Over Sea Extension to Key West, completed in 1912. Many of the gaps between the keys were partly or completely closed by fills and approaches to bridges, and the normal currents into and out of the basins were reduced. This caused more impounding of water, and more rapid sedimentation seems to have taken place. It would have been very interesting to have accurate charts of the shallows just before this construction and just before the great 1935 hurricane, which washed out many of the fills that had dammed off some of the previous gaps. It seems from all available comparisons that the mangrove islands grew faster during the period of the railroad, from 1912 to 1935, than during a similar previous time.

The effects of along-shore or onshore currents upon sedimentation are less obvious near other parts of the Florida coast. On the west coast there is an absence of prevailing currents, and the coastline is minutely irregular. Very local conditions of current, tide, and wind account for most of the sedimentation. The strong countercurrent along the east coast sweeps the sediments southward, and it, with the strong wave action, prevents the formation of offshore shallows favorable for initial mangrove growth. Beaches, dunes, and their strand flora are found along this coast, and mangroves grow only along and in the shallow estuaries, or other depressions back of the coast proper. There seems to be little or no extension of the east coast by mangroves.

Since the outer, mostly *Rhizophora,* swamp zones are nearly always continually submerged, the part played by molar factors does not cease with the advent of a swamp. If the prevailing current is along shore, sedimentation in the swamp is less rapid than if the current is onshore. If the tides are very regular, there is less sedimentation than if they are irregular with long slack periods for the settling out of materials. If the waters are shallow and cover fine sediments, strong onshore winds churn up these sediments and wash them into the swamps. The mechanical effect of the roots of the mangroves in reducing currents of whatever origin and causing the settling out of materials is the most important factor in the soil-accretion process. If the swamp is a narrow, inundated zone, with little opportunity for the water to become quiet, or if by a combination of high tidal range and waves the surface water remains agitated, there is less deposition of materials than in a broad swamp having only occasional exchange of water over the surface.

Without doubt the tidal range over these littoral swamps has changed locally with changing offshore conditions and other factors that affect the configuration of the coast. A reduction in the height and strength of the tide leads to settling of materials where they were previously shifted about. If a shallow basin becomes cut off or surrounded by bars, its tidal and other currents are reduced. Mangroves often begin around the basin and, with the aid of increased sedimentation, tend to help fill it in with organic as well as inorganic materials. Such a near-circular basin is the Marquesas Keys, which form an atoll. Fine muds are accumulating within it, and mangroves are extending inward over the muds. Many enclosed or nearly enclosed ponds now part of the present shores were probably small bays which had sufficiently wide openings to allow normal tide exchange, but which now have none. Basins such as Tarpon Basin, Lake Surprise, and Long Sound (fig. 6) are good examples of intermediate stages in the filling in of formerly more open bays. Many of the mangrove swamps have outer zones on higher soils than the inner zones, the latter covering the lower, last-filled part of the basin. However, some of the lower parts may be the results of depression of the soil level, forming paludal basins.

Tidal range and tide-level changes are of geological significance, for usually long periods of time are necessary. The deep deposits in many mangrove swamps indicate that the sea-level plane has changed, and the probable extent and direction of these changes are discussed in the section on "Geology and Coastal Changes."

It has been pointed out that most of the materials which settle out at low tide are again raised with the high tide, so that the net result is negligible. There is little doubt, however, that there is some consolidation of materials at low tide, increasing their mass, so that there may be some accretion directly due to the mere rise and fall of the tide. In some places quantities of fresh materials are brought into the littoral zone, so that this effect of the tide is of some importance.

In summary, the marine molar factors along the mangrove-inhabited parts of the Florida coast are very favorable for soil-accretion processes. Erosive action is reduced to a near minimum in the near-shore shallow waters. Most of the marine deposits would occur without the aid of mangroves, but mangroves accelerate the purely physical types of deposition by materially reducing currents, tides, and wave action.

In addition to the marine factors, river currents may be important. These are usually too weak to be very effective in distributing sediments over the swamps, but seasonal rains may alter this condition and cause strong currents. Some of the rivers from the Everglades carry large quantities of débris on their surface, and may be so greatly charged with finely divided organic material that they have a characteristic coffee color. As compared with other coasts, few inorganic sediments are deposited by rivers over the swamps, as the Florida mainland is in general so flat that little or no erosion occurs. Dissolved lime rock materials cause many of the rivers to be calcareous, and these carbonates may precipitate or settle out of the slack waters over the swamps. Some fine silts and muds cover the swamp soils after hard rains have swollen the rivers. Part of the plastic peat soils are derived from river-borne organic sediments, washed from interior marshes or swamps.

Mangroves along the shores of these estuaries are benefited by the sediments carried along by river currents. Just as in most rivers, the strong currents along the convex side of a sharp bend may tend to erode away soil from the swamps, whereas bars form in less swift parts of the stream, furnishing starting points for mangroves. The characteristic occurrence of numerous small mangrove islands at the mouths of rivers is due to the more abundant silting of materials where the river currents are arrested by tide and other marine currents. In many parts of the tropics the large mangrove swamps are confined to the mouths and banks of estuaries. Delta mangrove swamps are more common than any other type. Where the rivers drain high uplands, the sediments deposited by them form most of the mangrove soils, and the rôle of mangroves in building up soil levels is more related to river deposits than in Florida. This relation between river sediments and mangrove swamps is well recognized throughout the world, even though it is of little importance in Florida. Thus, in general, the absence of mangroves along dry tropical quiet-water coasts is due, not to the direct effect of dry conditions on the mangroves, but to the lack of rivers bearing sediments.

Storms, especially violent hurricanes, may be very effective in promoting soil accretions. Many of the deposits of marine soils far inland owe their origin to hurricanes. The high winds churn up the water so violently that it becomes heavily charged with sediments and organic débris. The high water is swept into the swamps and much of this material deposited. Although no measurements of the depth of such deposits after a hurricane were made, the presence of a definite marl layer over peat indicates some exceptional condition of high winds and waves. Much of the marl over the Cape Sable region seems to have been swept in from Florida Bay by storms.

Storms not only add to the soil materials in the swamps but also wash out soils, in some cases completely removing all the upper layers from the more exposed places. In some instances they erode away the soil of an island completely. Storms also change the configuration, position, and size of many of the submerged shoals and bars.

Physicochemical Factors

The physicochemical aspects of sedimentation are less important, more complex, and less well understood than the molar factors. Changes in salinity, variation of carbon dioxide content, hydrogen-ion concentration, carbonate and bicarbonate content, temperature, plant and animal life, all affect precipitation and the solubility of carbonates. The precipitation of calcium carbonate is the most significant result of certain combinations of these conditions, and many views of the fundamentals of this process have been studied. Although some of the details of such precipitation are important in all studies of deposits in shallow sea water, they need not be elaborated here, as they have been well presented by Twenhofel (1926), Revelle (1934), Thorp (1935), and others.

Marl, oölite, and other calcium carbonate soils are so abundant in southern Florida that physicochemical conditions are probably more important here than in most places along the coast. However, this precipitation in the swamp or shoal waters is negligible as compared with the quantity of materials that mechanically settle out or are shifted along the bottom. Thorp (1935), in his study of many samples of the shallow-water deposits of regions B and E of figure 1, found only 5 or 6 per cent of undifferentiated calcium carbonate not obviously assignable to organic origin as parts of calcareous algae, mollusks, corals, foraminifera, and other marine organisms. This undifferentiated calcium carbonate may be only partly of inorganic origin, as marine bacteria may be important, as shown by Drew (1914), Gee (1936), and others. Vaughan (1914*a*) in his discussion of the origin of the Floridan and Bahaman oölites states that bacteria are probably very active in shoal water and are precipitating calcium carbonate in the form of aragonite. Chemical precipitation of calcium carbonate may form spherulites which by accretion may become oölite grains.

A few general features of precipitation or solution of calcium carbonate are worth considering. It is probable that variations in hydrogen-ion concentration are more important than changes in salinity. The complex physical and chemical factors which determine the hydrogen-ion concentration are included under the term buffer mechanism of sea water. Carbon dioxide content, bicarbonate and carbonate ions, temperature, salinity, and many other variables affect this mechanism.

Revelle (1934) summarizes the factors affecting the solubility of calcium carbonate in sea water thus: "Except for water in equilibrium with the atmosphere, a condition probably only rarely established, the most important factor controlling the solubility of calcium carbonate in sea-water is carbon dioxide content of the water, which is chiefly dependent upon the nature and amount of biological activity." Thus the plant and other life of the swamps and shoal water is important in the purely physicochemical processes of precipitation.

The conditions which tend to promote calcium carbonate precipitation are high salinity, removal of carbon dioxide from the water, agitation of the surface water, and high pH values. In general, the shallow, open shoal waters abound in plant growth, have high temperatures and high salinity, are much agitated by winds, and have a high pH. Within the swamp zones the water is very shallow, but it is not often agitated, there are fewer submarine plants, the water is less salty, and the pH values are lower. Therefore, it seems probable that there is more calcium carbonate precipitation in the open shoal water than in the swamp water.

Extensive chemical tests could not be made and were not justified because of the relatively minor rôle of physicochemical precipitation. Only a very few water analyses of carbonate and bicarbonate content were made, but these indicate some conditions that may be significant. These were made in 1936 from regions B and C (fig. 1), and table 16 summarizes the data. The outer, deeper-water parts of each region are compared with the inner, shallow, shoal-water and swamp-water parts of the same region. In region B the bicarbonate content and salinity both decrease from the outer to the inner parts, but the total carbonate content shows no regular decrease. In region C bicarbonate ions were found only in a few samples along the outer side of the Ten Thousand Islands. In region C as in region B, salinity decreased inland, and there was no marked decrease in the total carbonate. The general presence of bicarbonate ions in the deepest, open-water parts, whereas little or none is found in the shallowest, shoal- or swamp-water parts, indicates more absorption of carbon dioxide in the former parts, but since total carbon dioxide content was not measured, this condition is inferred. Some pH values were obtained, and these few indicated higher values in the shallow-water parts. Since 1936 was an abnormally wet season, the salinity was less than normal. During most seasons salinity increases somewhat in the shallow-water parts of the outer swamp zones because of evaporation. All these conditions should be measured more accurately in future studies, for some more

TABLE 16. *Averages of water analyses for bicarbonate and carbonate content and salinity, outer and inner parts of regions B and C (fig. 1), summer of 1936*

Region and part	No. samples	Carbonate (p.p.m.)	Bicarbonate (p.p.m.)	Salinity (per cent)
Region B:				
Outer	17	138.7	10.20	3.676
Inner	36	146.6	7.67	2.553
Region C:				
Outer	9	126.5	7.48	1.293
Inner	15	123.0	0.00	0.365

exact correlations between swamp zones and precipitation of sediments might be found.

Precipitation of silts and muds due to the action of electrolytes may be even more important than calcium carbonate precipitation. Where sea water and fresh water mix at the mouths of rivers and other places along the coast, there is much sedimentation that is not all purely mechanical. Large quantities of fine materials are deposited because of the action of electrolytes associated with certain types of mineral colloids, causing the flocculation of some of the finely suspended matter. Since humic materials, which are most abundant in the swamps, are not easily agglutinated, there is probably less sedimentation due to this flocculation in the swamps than in the open water. The amount of this type of sedimentation was not calculated because it is almost impossible to distinguish it from the purely mechanical settling out of materials.

BIOTIC FACTORS

Most of the organic débris that accumulates on top of, interbedded between, or mixed with marine sediments is of mangrove origin. The partly decomposed remains of the mangroves and associated plants form the fibrous peat, which has few inorganic constituents. Much of this peat is derived almost entirely from *Rhizophora* or *Avicennia.* The sedimentary peat and the plastic marl, shell marl, calcareous sand, and silicon sand soils all contain less of this definitely mangrove material.

In the discussion of soils and their chemical composition and in the discussion of soil profiles the importance of the soils of high organic content was stressed. Plants contribute more than animals to the accretions, but both are important.

In general, brackish-water plants grow more rapidly than fresh-water plants and their decomposition is retarded. Decomposition is least in situations of high salinity, although there is decreased plant growth. Thus, quite contrary to what Bowman (1917, p. 666) thought, the débris in these saline swamps does not quickly decompose into mud and soil. As in bogs, the slowness of the decomposition is due to a number of factors, among which lack of much oxygen, acid nature of the plant material, and salinity of the water are probably important.

The plant populations of each zone of the swamps primarily determine the nature, depth, and rapidity of accumulation of organic deposits. The materials washed in from the sea and from the adjacent inland are usually less than those derived from the swamp itself. Along the outer edge of the swamps, broken parts of marine aquatics, particularly *Thalassia* and *Cymodocea,* with parts of algae are washed into the swamps in large quantities. The plastic sedimentary peat soils also owe their origin, in part at least, to fine organic materials washed in from adjacent marshes.

The associated animal life contributes much to the calcium materials, the mollusks, foraminifera, crustaceans, sponges, and worms being most important. The animals are in general much less abundant in the interior of a swamp than along the edge, or in the adjacent offshore water. In most instances basal soils of marl, calcareous sands, shell, and coral fragments are dominantly of animal remains. The upper soils, those accumulated after the mangroves have formed a mature swamp, are dominantly of plant remains.

Besides animals, calcareous algae contribute much to the calcareous sands, and the possible contribution of bacteria has been mentioned.

GEOLOGY AND COASTAL CHANGES

The Floridan Plateau, of which the present peninsula of Florida is only a part, extends some distance westward into the Gulf of Mexico and a short distance southward to the deep Strait of Florida. This plateau has a geologic history similar to that of the south Atlantic and Gulf coastal plain. The present land areas are more extensive than they were during parts of the Pleistocene epoch and less extensive than during other parts of the same epoch. Such an increase and decrease in land area has been variously interpreted by geologists. Some hold the view that there have been uplifts and depressions, but the interpretation of Cooke (1935), that the levels of the Pleistocene shore lines are the results of changes in the sea level, seems more plausible. His views are based on the influence of polar and subpolar ice caps on the sea level. He states (1937), "When the ice caps are large there is less water in the sea than when the ice caps are small." He estimates the height of the Pamlico formation near Myrtle Beach, North Carolina, during the Peorian interglacial stage as 25 feet above the present level, but he gives no estimate of the low level of the Wisconsin stage, or of the increase in level since that stage.

The account given by Cooke (1939) of the Talbot, Pamlico, and other late Pleistocene stages in Florida indicates that the fluctuations in sea level can be traced with a fair degree of accuracy over the Florida peninsula. Most of the regions south of Lake Okeechobee, including the Everglades, Big Cypress Swamp, and the mangrove regions of Florida Bay and the southwest coast, are parts of the last, or Pamlico, terrace. If, as seems the rule, a low sea level is followed by a rise in sea level, then the appreciable drop in sea level since Pamlico time has probably been followed by a rise in sea level since this last low stage of water. At least a small rise seems to be indicated by the peat deposits in some of the older mangrove swamps.

The pre-Pleistocene crustal movements were without benefit of sea-level changes from the effects of glaciation. How much change in shore lines has taken place in Pleistocene and recent times is fairly well known. A few evidences besides the terraces indicate a slight recent rise of the sea level in southern Florida and the coral-reef tract. The present coral-reef tract seems to be built on an older coral bed that was well below the present level. On the other hand, the exposed coral rock of the upper eastern Florida Keys was of course at one time below the water. The present level is between these two extremes. The evidence of a rise in the sea level from an interpretation of mangrove vegetation is at present less valid than the above, but may become more significant with further study.

Cooke and Mossom (1929), Vaughan (1914*b*), and Vaughan and Shaw (1915) give further accounts of the geology of the Florida peninsula that include references to uplift and depression of the land during periods before the Pleistocene. In the late Miocene, central Florida was above the present sea level, and since that time the dome-shaped axis of the peninsula has stood above sea level, but the flanks have slowly oscillated up and down. Sea-level changes may have occurred also but are not well indicated until Pleistocene glaciation. There is no evidence that mangroves contributed to coastal soils during glacial times, but only after the last glacial period. It is, therefore, only during Recent time that mangrove-soil deposits may be considered important in estimating coastal changes or interpreting oscillations in sea level. Students of salt-marsh peat deposits have considered them indicative of changes, and similar interpretations may be developed for mangrove deposits.

Two methods of salt-marsh formation are recognized by Chapman (1938). The first of these is the formation of marshes during a gradual subsidence of land or rise in the sea level, with silting or organic accretions taking place at a greater rate than the change in level; peat beds much deeper than the tidal range could not be formed without such subsidence or rise. The second method is the silting-up of shallows protected by bars without any accompanying change in the tide level. In this latter case the thickness of the deposit will be nearly the same as the maximum tidal range (Nielson, 1935). The first method of interpreting salt-marsh soil deposits may be applied to the mangrove-swamp soils in Florida.

In the preliminary discussion of the geologic rôle of mangroves it was pointed out that the mangrove soils and plant zones furnish a basis for the interpretation not only of present accretions of geologic importance, but also of past levels of the sea. Such accurate estimates of the age of these mangrove swamps as those by Chapman (1938) for salt marshes are at present impossible, as many more data are needed on soil depth and stratification. It is, however, evident that some interpretations of former conditions can be made from these preliminary observations and data.

The deep peat soils of the southwest Everglades coast, and at some favorable localities around Florida Bay and along the Florida Keys, can hardly be explained as the result of accretion within the present vertical limits of mangrove growth. Since mangroves begin growth on soils below the lowest tide levels and may grow on soils a little above the highest tides, the swamp deposits might be somewhat thicker than the tidal range without any evidence of changes in the tidal plane. But many of the soils are three or four times as thick as the present tidal range. Therefore, either the range of different tidal levels has materially changed locally, or there has been a gradual rise in the sea level since these mangrove swamps began. No doubt local differences in tidal range have occurred, for some places, such as Florida Bay, now have a range of only a few inches while others have over 5 feet. There is no good evidence, however, that the general range was formerly wider, and the deepest deposits are deeper than any probable recent range. A general rise in the sea level is therefore indicated.

A 10-foot deposit of fibrous peat in a region with a present tidal range of 3 feet indicates that there has been a change in level of about 7 feet. As 14-foot deposits are found where the maximum tide range is about 4 feet, the change of level there would be about 10 feet. Similar rough calculations of the difference between depth of peat deposits and present tidal range at ten stations in mature swamps of regions B and C (fig. 1) indicate an average change in level of 7.4 feet for the southern and southwestern Florida regions and a few of the Florida Keys.

Some of the swamps are obviously much older than others and thus record changes in level over a longer period of time. The most recent swamps have no peat deposits. Since at present the rate of deposition in these swamps is not known, no detailed estimate of their age will be attempted, as has been done in some marsh regions. Where new swamps have started over submerged areas that were shown on old maps as bare of emergent vegetation, there is not enough peat or muck to give a good estimate of the rate of deposition. Marl by itself, or with peat, may fill in a swamp so rapidly that soil levels change appreciably in a short time. Most of the insular and coastal land-area increases are in regions where marl is abundant in the deposits. The deep-peat areas have changed little in outline on the maps, thus indicating that peat accumulates much more slowly than marl.

There thus appear to be reasonable evidences of sea-level instability with an appreciable rise, perhaps to a maximum of 10 feet above the level when the oldest mangrove swamps began, which was after the last glacial period.

Only the assumption of a slowly rising sea level can explain how mature mangroves could grow to the very edges of deep peat deposits facing bays and estuaries 6 to 10 feet in depth, for seedlings do not begin growth in water 6 or more feet deep. Furthermore, the seaward side of these swamps contains no deep organic deposits of plant material that might have washed out from the swamps and served as shoals for a pioneer colony of mangroves.

Many paludal basins indicate that settling has accompanied deposition of peat. Also changes from *Rhizophora* to *Avicennia* peat, then back to *Rhizophora* peat, are indicated in some of the soil profiles. These things should be considered as possible indications of periods of relatively stationary sea level, or even lowering of sea level, both affecting estimates of rise in sea level.

The above general interpretation of deep mangrove deposits as indicating a rise in sea level is entirely a preliminary suggestion. A gradual sinking of the coast would explain the deposits as well as a rise in sea level, but, awaiting further evidence, the latter is accepted as the most plausible explanation of the cumulose soil conditions.

Although the effect of the rock substratum on the mangrove vegetation is unimportant in most places, it is important along parts of the Florida Keys and in some other regions. The calcareous nature of the Miami oölite limestone region has a pronounced effect on the vegetation, since much of the marl of the whole southern region may be derived from this source. The large areas of pineland and some of the Everglades Keys hammocks are on this rock, and mangroves grow adjacent to these communities. The chain of the Florida Keys has two types of rock, the effect of which is especially significant. The eastern keys, which terminate in Loggerhead Key, are long, narrow islands composed of Key Largo limestone containing coral heads, and were formed from coral reefs that grew at the edge of the water; the western keys, which extend beyond Key West, were formerly shoals with a basic Miami oölite limestone rock. On the higher parts of the eastern keys are tropical hardwood forests that often extend to the water's edge. Thick mangrove forests cover only the tidal parts of these keys, and grow mostly in deep peat soils or in pockets of the porous rock. The vegetation of the western keys is quite different, with pinelands predominating on the higher portions and sparse, scrubby mangrove growth common over mud-covered tidal flats.

The paleontology of the mangroves themselves is fairly well known. Darrah (1939) states that *Avicennia* has been recorded from the Miocene and Pliocene of Trinidad, and *Rhizophora* has had an unbroken history since the Eocene in Georgia. *Conocarpus* and *Laguncularia* made their appearance in the Eocene. It does not seem, however, that the Florida mangroves have occupied the present littoral belt for a long time, as coastal changes have been too great. It is improbable that deposits of mangrove peat older than Recent times will be found along these coasts, but if found, such deposits may prove to be of paleontological significance. It may be that mangroves have formed or will form peat beds of sufficient thickness to be preserved as coal deposits.

SUMMARY AND CONCLUSIONS

The mangrove swamps of the low-lying coasts and islands of central and southern Florida were studied during five seasons to determine the ecology of these unique littoral swamps, and to obtain some idea of their importance as geologic agents in extending the coasts and forming islands in the shoal-water regions. Five coastal and insular regions of the peninsula were selected and a number of stations established in each for observations and experimental studies.

The report is divided into two parts. The first, "The Ecology of Mangroves," is concerned with the types of plant communities of the mangrove and associated vegetation, the successional relationships of some of these communities, the major environmental factors in and near the swamps, and the dispersal and establishment of some of the peculiar viviparous mangrove seeds and seedlings. Some descriptions of the experimental plantings are given.

The second part, "The Geologic Rôle of Mangroves," considers the accretions of sedimentary and cumulose soils in connection with the different agents that bring them about, and, more significantly, the rôle of the different mangrove communities in forming soils at higher and higher levels. The evidences of land-area changes are given, and the geologic significance of some of the coastal and insular changes discussed. Some of the geologic history of the region is given, and the probability of changes in the sea level since the last glacial period, as indicated by the deep peat deposits, is discussed.

The mangrove and associated vegetation consists of a number of distinct zones or belts more or less definitely related to water level and degree of salinity of the surface and soil water. Some of these communities occur in a successional trend from offshore pioneer communities to upland, or fresh-water, non-halophytic communities inland from the mangrove swamps. The most apparent succession of the mangrove communities consists of: (1) a pioneer Rhizophora family, usually associated with a marine aquatic associes on submerged shoals; (2) a mature Rhizophora consocies, as an outer zone along the coast, or completely covering young islands, both of which are flooded by salt water; (3) an Avicennia salt-marsh associes, not always flooded by salt or even brackish water; (4) a Conocarpus transition associes, seldom if ever flooded by water; (5) a tropical or semitropical forest association, which is the actual climax of the region.

The first two of these communities are composed of the dominant red mangrove, *Rhizophora mangle,* and very few or no other woody plants. With the pioneer community are marine algae and marine aquatic seed plants. This pioneer stage matures into a forest swamp composed almost entirely of medium-sized and large red mangroves growing with interlocking arched prop or buttress roots. These roots form tangles that not only hold the plants in the loose and shifting soils, but also act as natural weirs, catching sediments and débris.

The third community, of *Avicennia nitida,* the black or honey mangrove, with certain typical salt-marsh plants, is varied in composition with decided local differences in the associated flora. *Batis maritima* and *Salicornia perennis* are the most constant salt-marsh associates, but *Sporobolus virginicus, Monanthochloë littoralis,* and *Spartina alterniflora* are locally abundant. The black mangrove trees usually form open stands with the salt-marsh plants between them. The quill-like, erect pneumatophores of the trees often cover much of the floor of the forest, extending above the usual high-tide level. This mangrove zone may be inundated by every average tide over its outer part, but may be free from average tide coverage over much of its inner part.

The mature Rhizophora consocies and the Avicennia salt-marsh associes are the two main zones along most coasts.

The white mangrove, *Laguncularia racemosa,* does not usually form a distinct community, but grows as a secondary species with either of the other mangroves in their respective swamp zones. In some cases a Laguncularia consocies is formed, usually occurring landward from the Avicennia salt-marsh associes.

The buttonwood, *Conocarpus erecta,* a non-mangrove, forms, with a great variety of small trees and shrubs, a transitional zone between the two main mangrove communities and some inland, non-halophytic type of vegetation. Many of the associated species are tropical hardwoods and strand plants. In some instances these are numerous and form an incipient forest of the hammock type common in southern Florida. Some of the mature tropical or semitropical hammock forests adjacent to mangrove swamps are now growing on soils of mangrove origin and are, therefore, the climax of the mangrove halosere. In other instances the non-halophytic type of vegetation inland from the saline or brackish-water swamps consists of fresh-water marshes or marl prairies, some of which are also growing on soils of mangrove origin.

Other mangrove communities are recognized and described, and their probable rôle in a succession noted. Of these, the mature mangrove forest associes of the southwest Everglades coast is the most striking, consisting of a tall, mature stand of all three species of mangrove on deep peat and muck soils extending many miles inland along the numerous tidal estuaries, and finally giving way to hammock forests, marshes, or swamps of fresh-water habitats. This is the largest mangrove region in Florida and one of the best in the world. It might be of great economic importance with proper silvicultural management.

Of the external environmental factors, salinity of the water and soil, tides, water level, and temperature are the most important. There is a fairly definite correlation between the zones of the swamps and degree of salinity of the water and soil, and also the water level. But there is not always a distinct tidal fluctuation in some of the mangrove swamps where zonation occurs, so that tides are not necessary for zonation. Mangroves are killed by severe frosts and the swamps are confined to coastal regions only rarely visited by light frosts.

The soils of the swamps are of three main types: (1) peats and mucks of cumulose plant materials with different proportions of sedimentary materials; (2) calcareous marl soils of small particle size, derived from oölite limestone, coral, and many submarine organisms; (3) sands and coarse materials of either siliceous or calcareous type, or mixtures of both. The nature of these different soils does not seem to affect the type of mangrove community as much as their layering indicates the manner of accretion within the swamps.

Mangroves have viviparous or partly viviparous fruits and seeds, and their seedlings or seeds are water-borne, so that their dispersal and establishment are very efficient. Studies were made of their direction and rate of dispersal between Key West and the Tortugas Keys. The manner of floating and sprouting of the seedlings, and of their establishment, were carefully studied. It seems probable that *Rhizophora mangle,* and perhaps other species, may travel more than a thousand miles and become established where there is not much wave action or shifting of the soil. Marine currents, tides, and winds are all effective in their dispersion.

Plantings of all three species were made on the different soils and in different depths of water. Some of these plantings have proved successful and are beginning growth into swamps, thus affording a basis for future observations on the development of both the communities and the soils.

The second part of this paper considers the numerous evidences of land-area changes, especially along the southern coast. The best evidences of these changes, mostly increases, were obtained by comparing the recent, accurate surveys of the U. S. Coast and Geodetic Survey and air photos by the Army Air Corps with some of the former surveys. Numerous instances of new and enlarged islands and some instances of extended mainland coasts were found. Many of these new areas were visited. If new, mature swamp areas are considered land, then there are more than a thousand acres of recently formed land in the Florida Bay to Biscayne Bay region.

The building up of the soil levels in the swamps was determined by many soil-profile studies at nearly all stations. Although different profiles have soils of different materials at similar levels, it is in general true that the cumulose soils of peat or muck are found above the basic soils of marl or sand. The peat often contains enough undecomposed material to permit determination of its origin. In many other cases the origin could be determined from general morphology, chemical analysis, and microscopic inspection.

The distinctly layered profiles best indicate the mode of accumulation of soils in the swamps. The development seems to be: (1) A basic soil upon which the pioneer Rhizophora colony began growth on a submerged shoal. Such soils are of marine origin and most often are the results of deposition by marine agencies of tide, littoral currents, and waves. (2) An intermediate layer of organic cumulose soils varying in composition from raw fibrous peat to a sandy or marl muck, and derived from the long-time accumulation of plant remains in quiet waters of the inundated outer zones of the swamps. This layer may be very shallow or very deep, depending upon the age of the swamp. (3) A top layer of sedimentary soil, marl or sand, washed in over the cumulose organic layer, over the inner zones of the swamps and above the present high-tide level.

None of the above layers are distinct in some of the swamps even though they seem to have been growing at their present location for a long time, and many swamps are of such recent growth that no sedimentary or cumulose soils have been built up in them.

The geologic importance of these built-up and extended coastal soils is not great, but the probability of continued development is very significant. It may be possible to use mangroves, by means of plantings, to extend the coast and form islands. In any case, the swamps are useful as protecting the shores from excessive erosion.

The deep peat deposits may be used to interpret probable sea-level changes since the last glacial stage. The deepest deposits are best explained by assuming a gradual rise in sea level since the beginning of the older swamps. The present depth of many of the deposits greatly exceeds the present, and probably the past, tidal range. Further study of these peat deposits may give more accurate information about coastal changes and prove to have significance similar to that of studies of peat deposits in salt-marsh areas.

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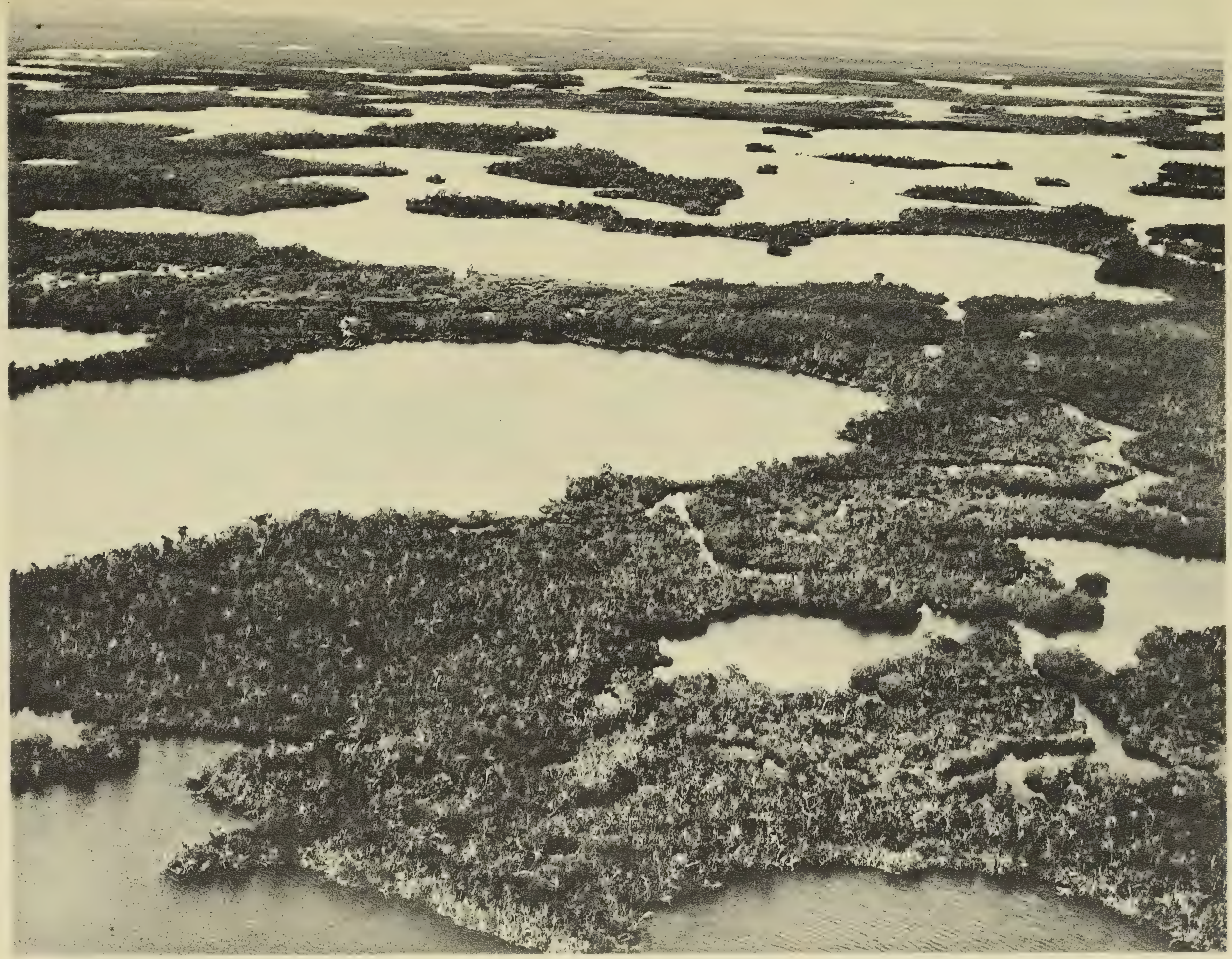
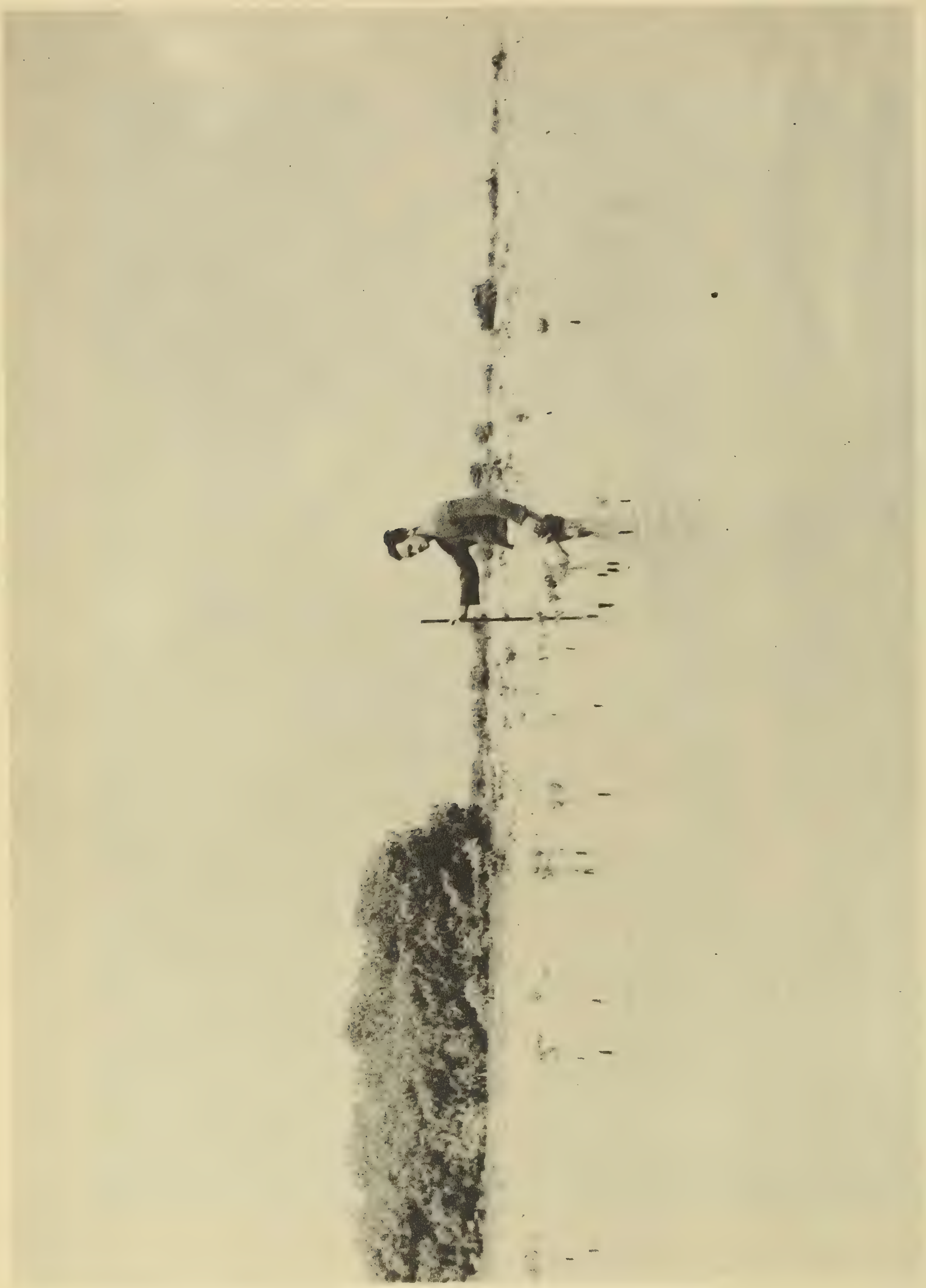


Air photo of the Shark River–Whitewater Bay region north of Cape Sable. Note the many small mangrove islands, some of which will grow together to form larger blocks of land.



A typical pioneer Rhizophora family on a marl mud shoal, Park Key shoal in Florida Bay.

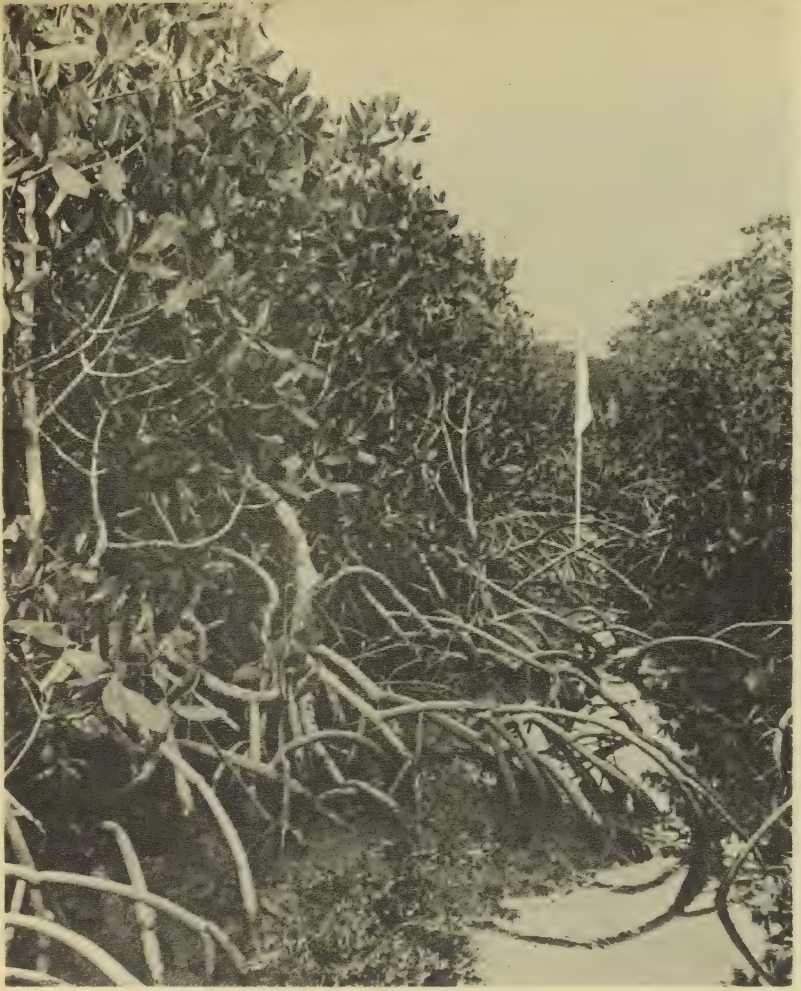


FIG. 1. Interior of a mature Rhizophora consocies swamp at low tide. The pole with flag is 7 feet tall. The fibrous peat soil here is 5 feet deep over coral rock.

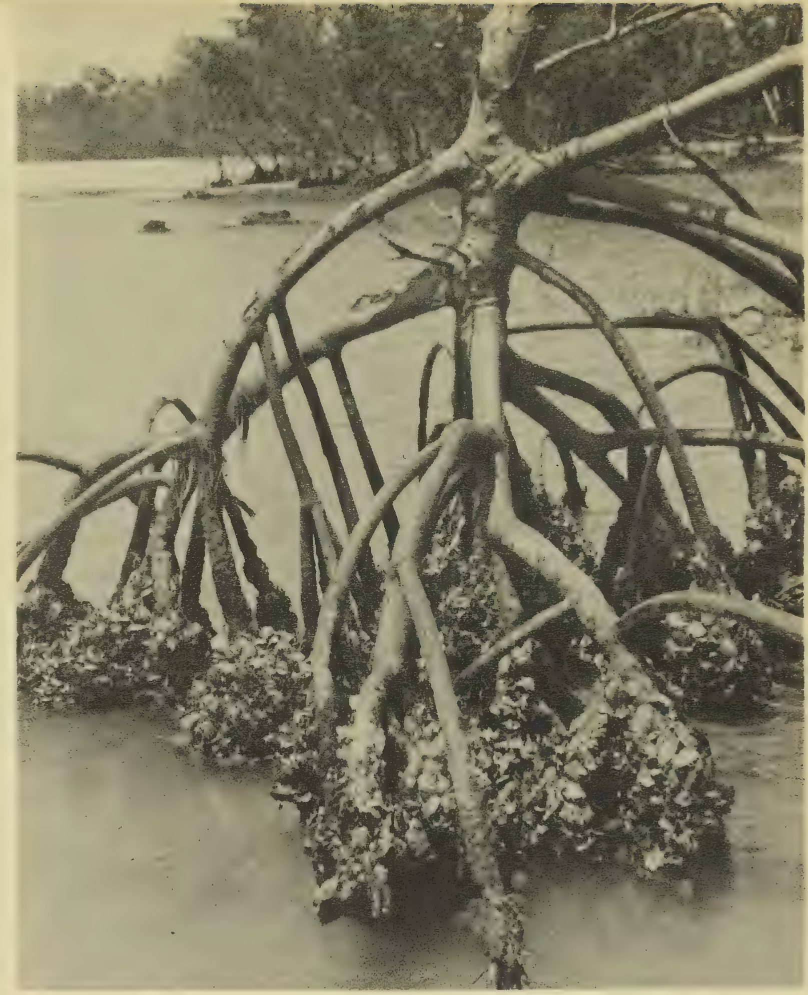


FIG. 2. Mangrove roots with growth of small coon oysters, *Ostrea frons*, on account of which mangroves are often called "oyster trees." Photograph taken at low tide; the oysters are covered at high tide.

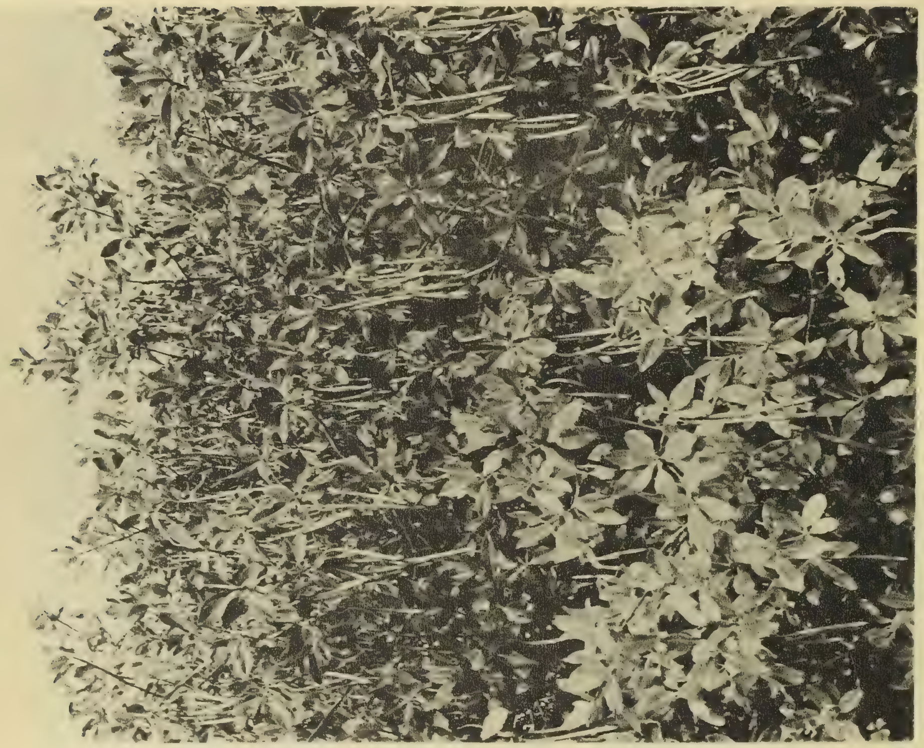


FIG. 1. *Rhizophora* trees loaded with nearly mature long, pendent viviparous seedlings.

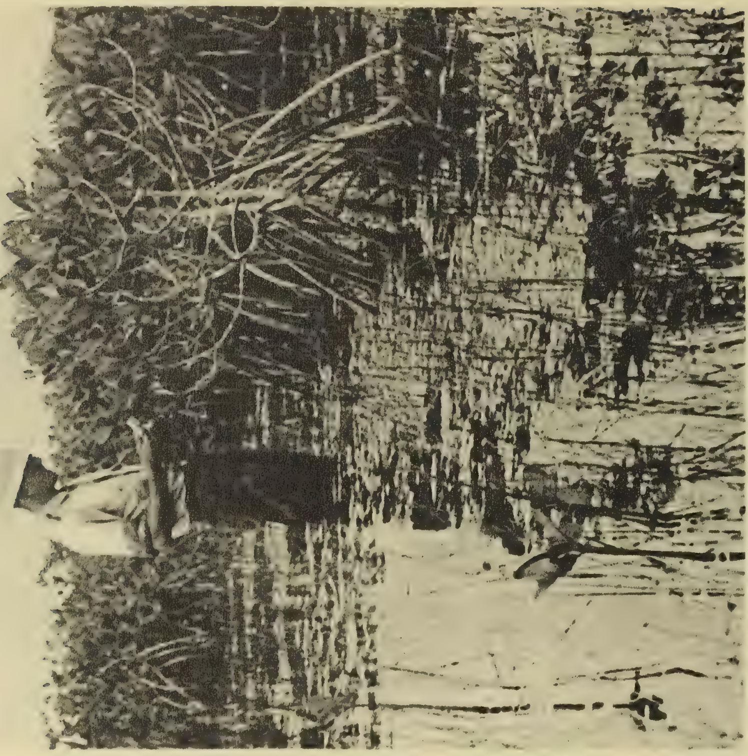


FIG. 2. Dwarf form of *Rhizophora* growing in nearly fresh-water part of the Everglades

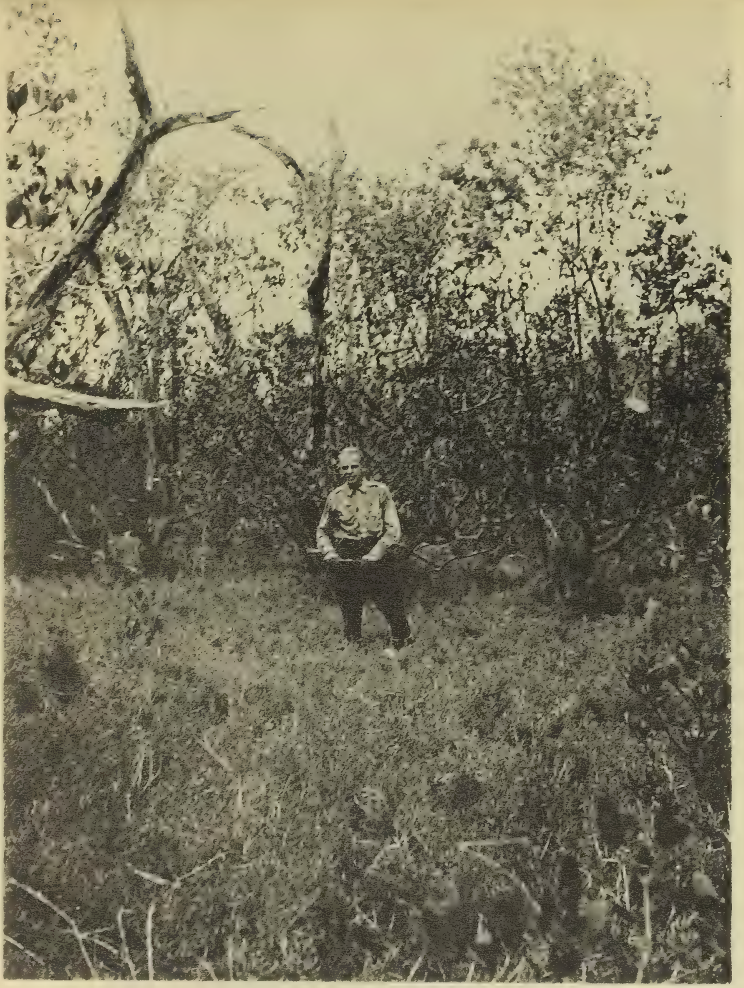


FIG. 1. Interior of Avicennia salt-marsh associes. Marsh plants in foreground mostly *Batis* and *Salicornia*. The assistant has just driven the soil sampler through 10 feet of fibrous peat and marl.

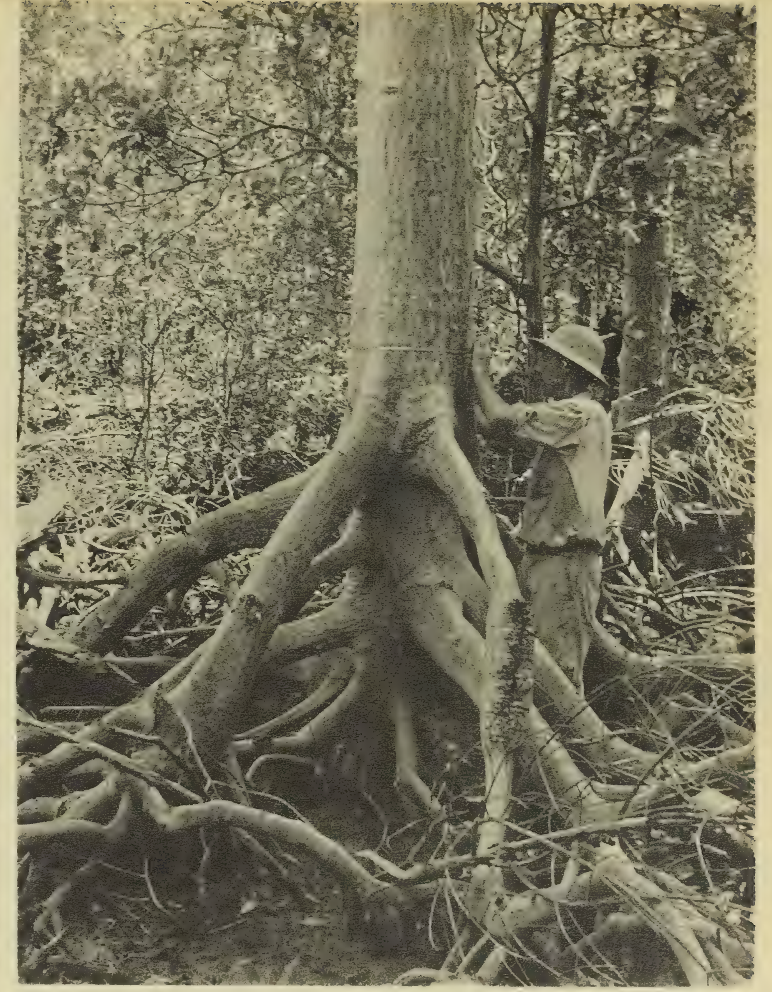
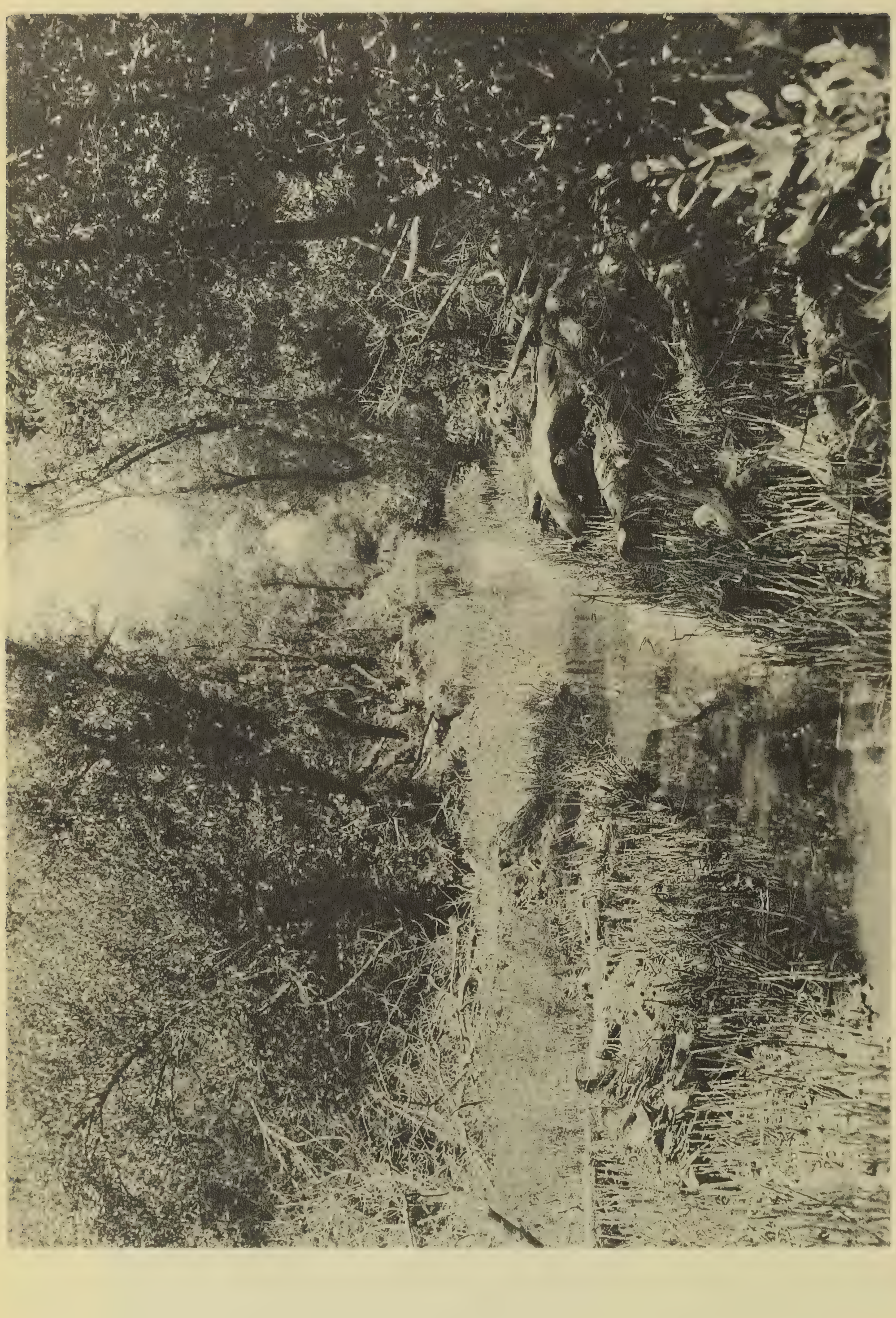
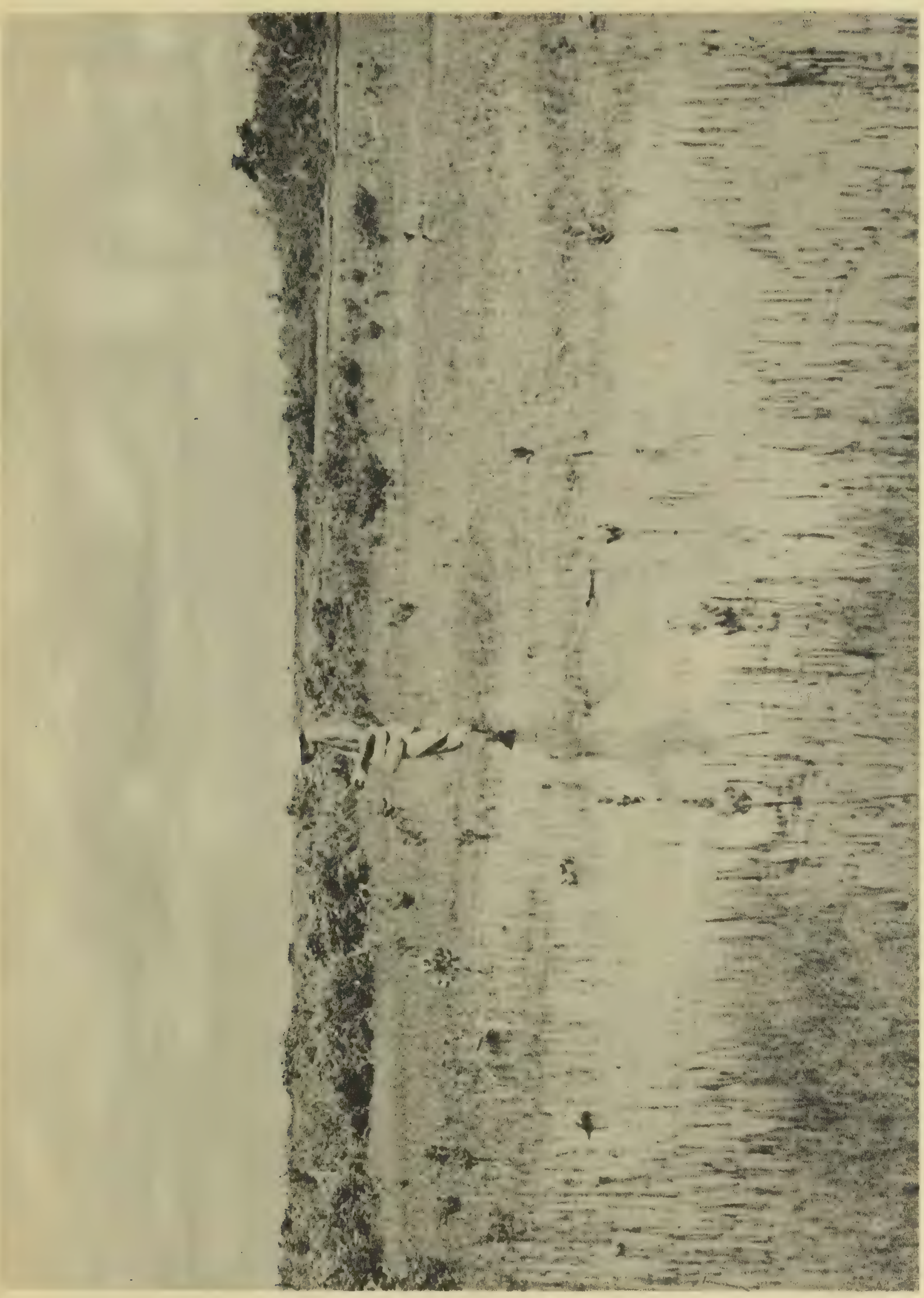


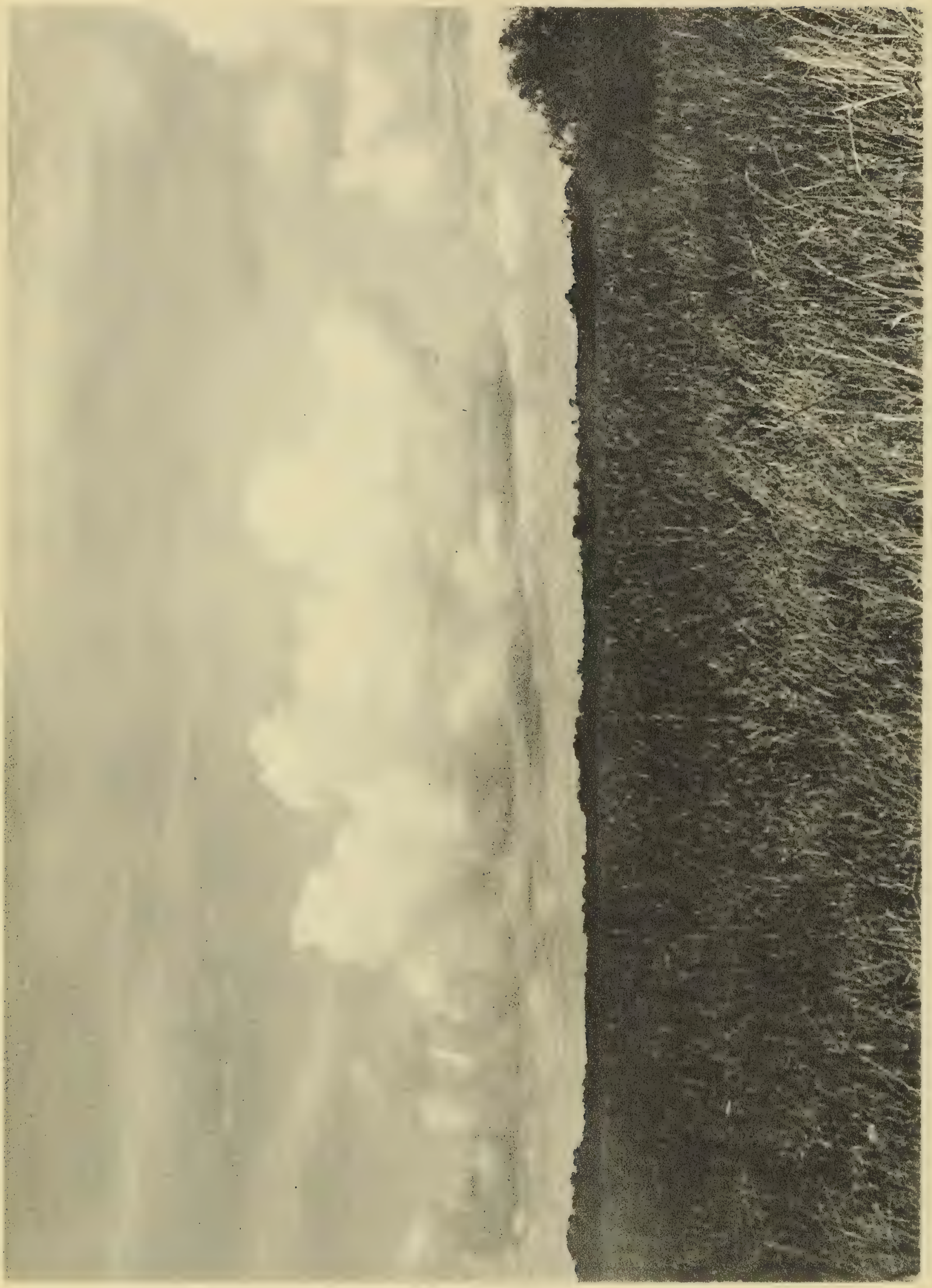
FIG. 2. Measuring a large *Rhizophora* of the Shark River region. Photograph taken at low tide. A high tide will be about 3 feet above the soil. The soil here is 12 feet of mangrove peat.



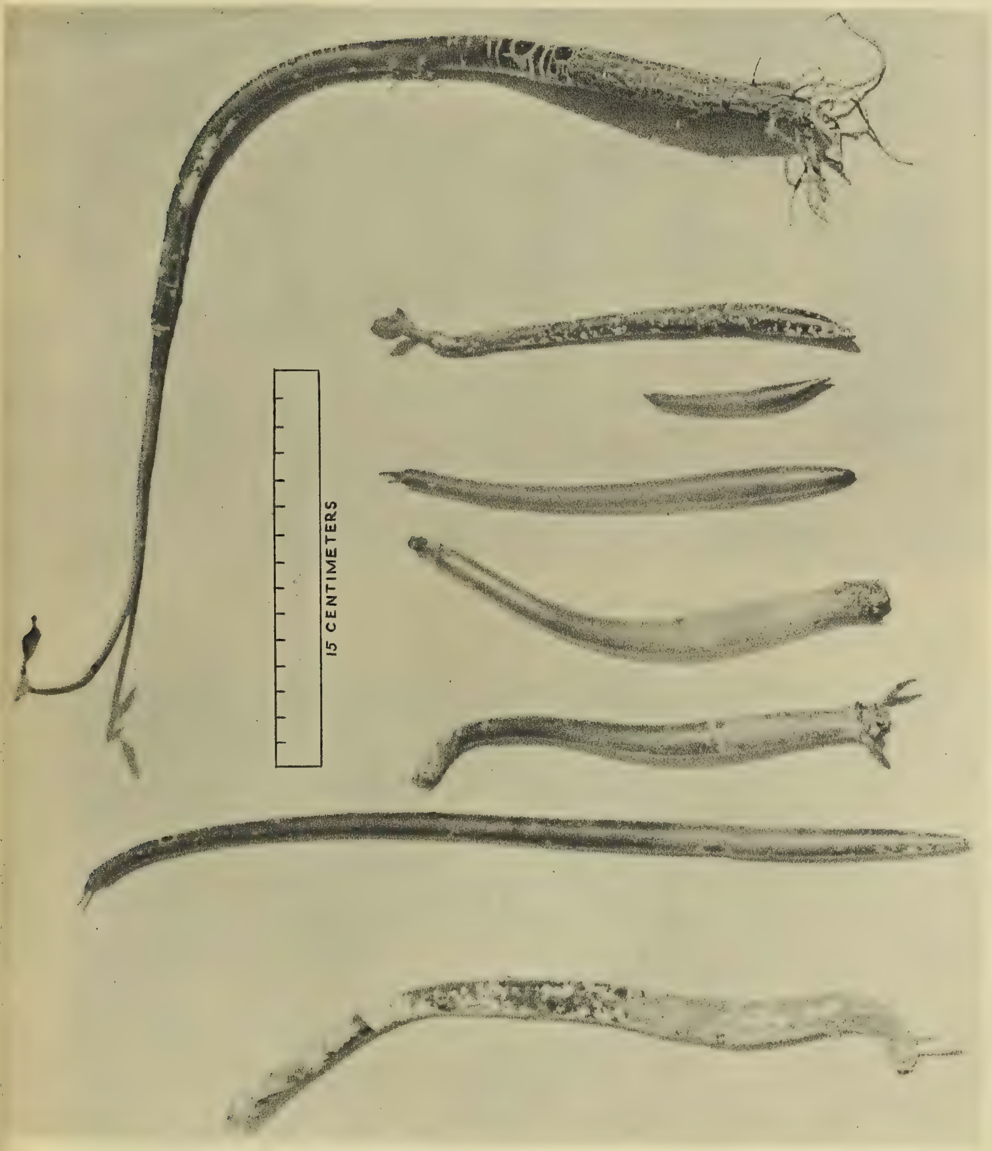
Drainage ditch through a black mangrove forest, showing abundant pneumatophores. There are few or no salt-marsh plants in such thick stands. (Courtesy of U. S. Department of Agriculture Bureau of Entomology and Plant Quarantine.)



Scrub mangrove zone between Avicennia associes and Conocarpus transition associes; a marl mud flat with salt-marsh plants. Buttonwood and hammock plants in background. Soil partly flooded by rain water.



The Everglades



Typical seedlings of *Rhizophora mangle* collected on the beach at Loggerhead Key of the Tortugas. The one on the left has been in the water a long time. The one next to it is recent, since its plumule has not unfolded and no small roots have developed. The large one on the right was well rooted and had over 6 inches of top growth before being uprooted.

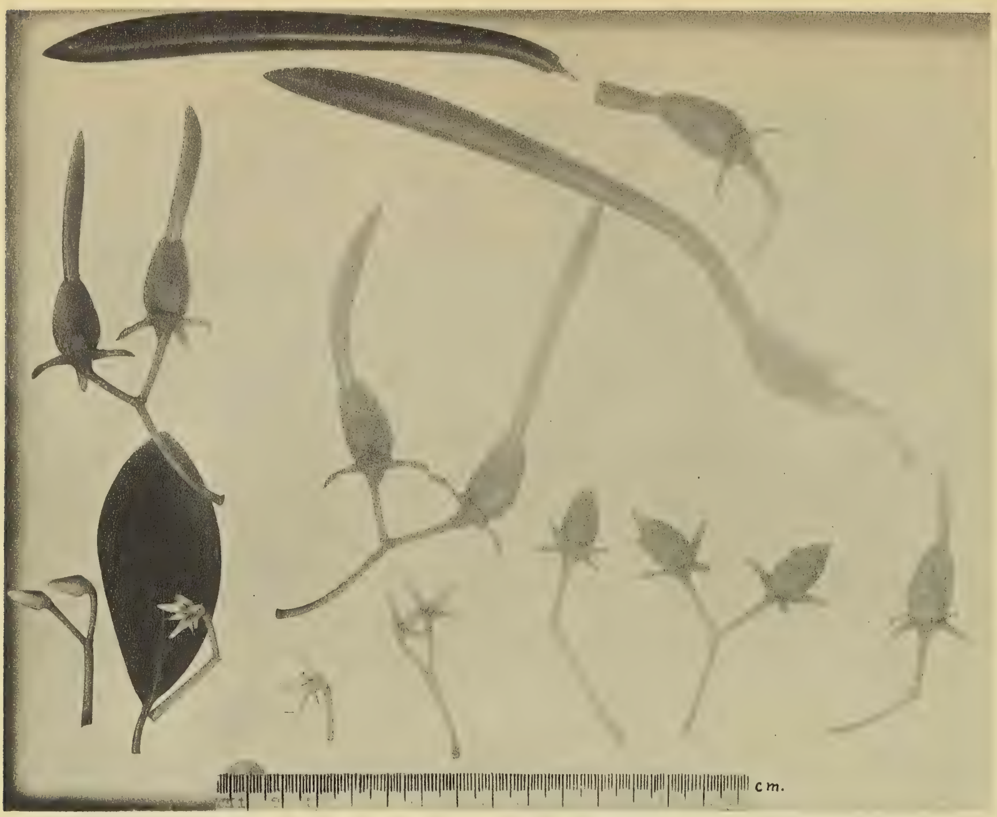


Fig. 1. Typical stages of the development of the viviparous fruits of *Rhizophora*, from flower to shed seedling.

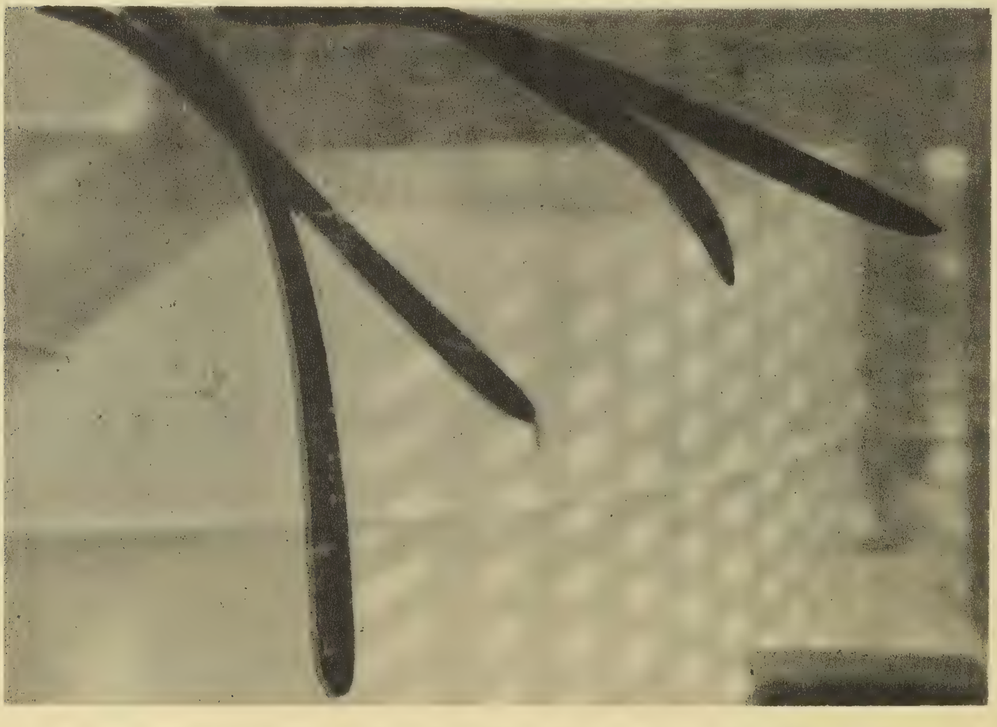


Fig. 2. Typical positions assumed by *Rhizophora* seedlings while floating. The position tends more and more toward the vertical with age.

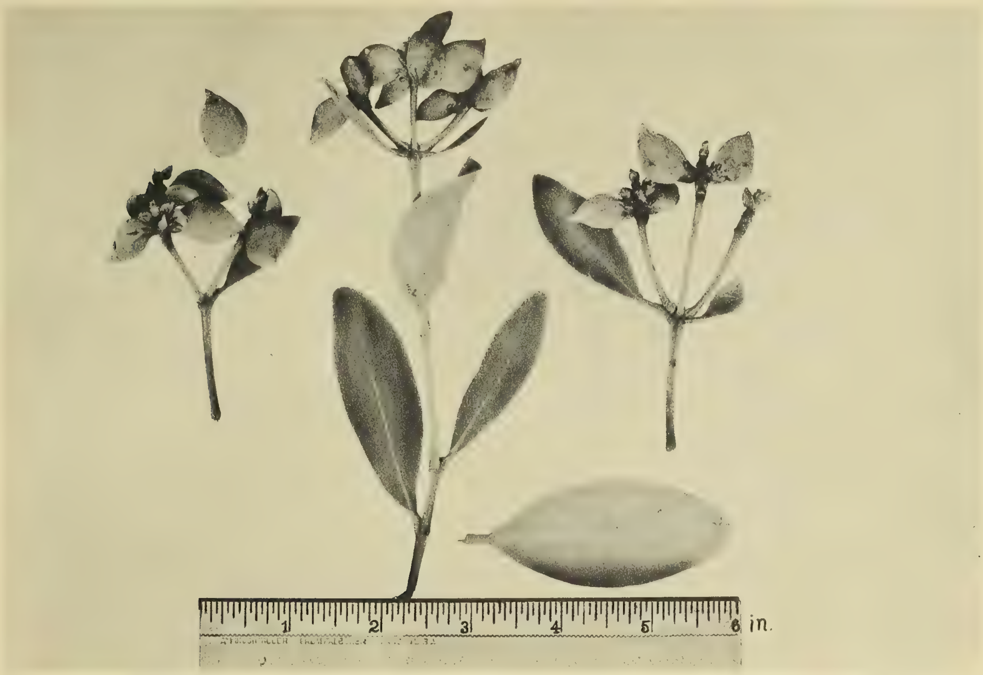


FIG. 1. Branches of *Avicennia nitida* with nearly mature seeds. Leaves pale and pubescent on under surface.

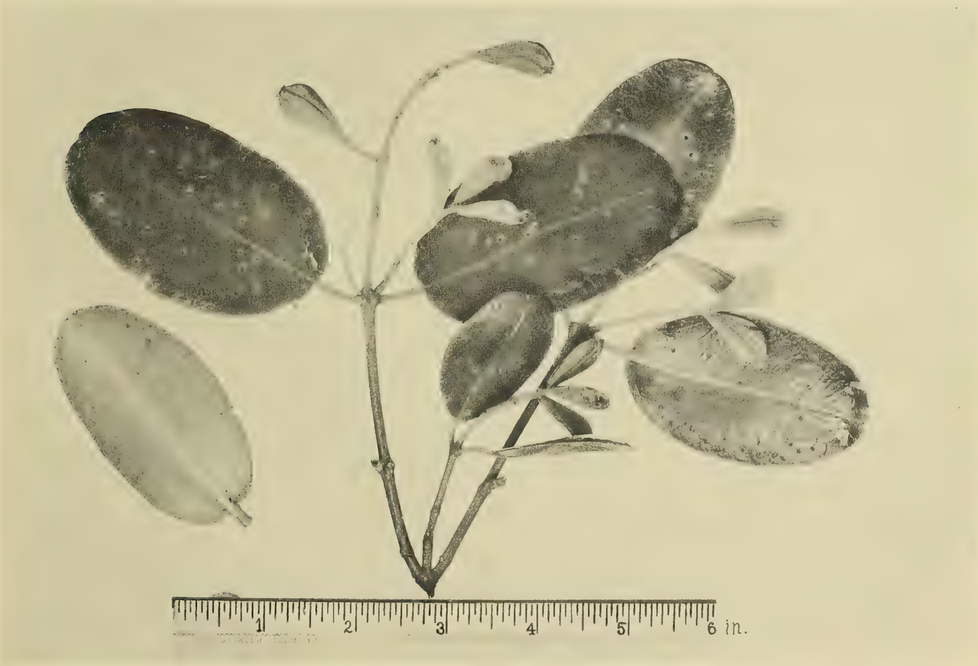


FIG. 2. Branches of *Laguncularia racemosa* with nearly mature seeds. Leaves thick and pitted, alike on both surfaces.

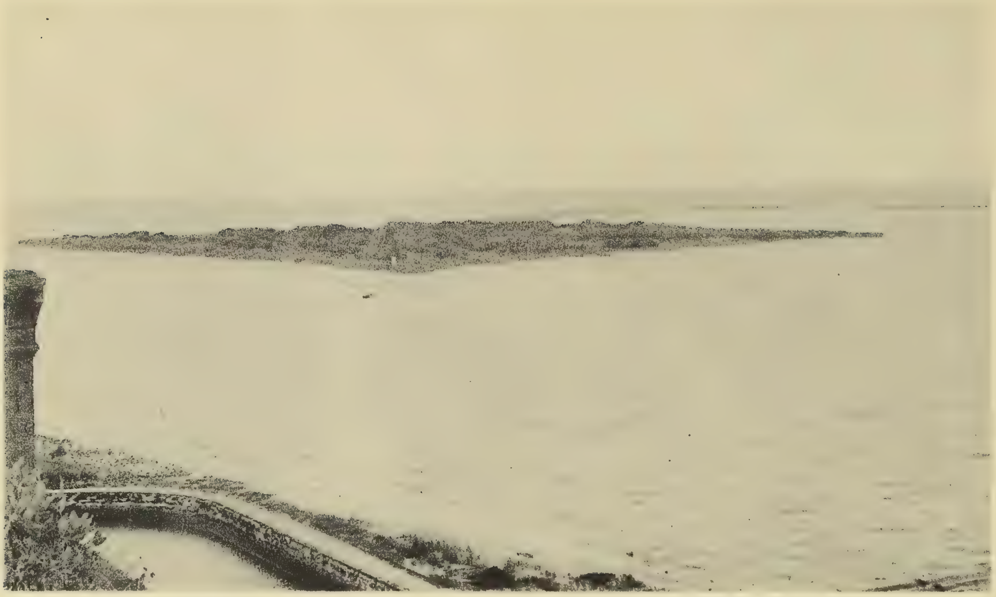


Fig. 1. View of Bush Key, middle foreground, and Long Key, background, from Fort Jefferson at the Tortugas. Most of Bush Key has been built up since 1907. Interior ponds on this key have mangroves. Over 4000 *Rhizophora* seedlings were planted on Long Key, and most of them have survived after 18 months.

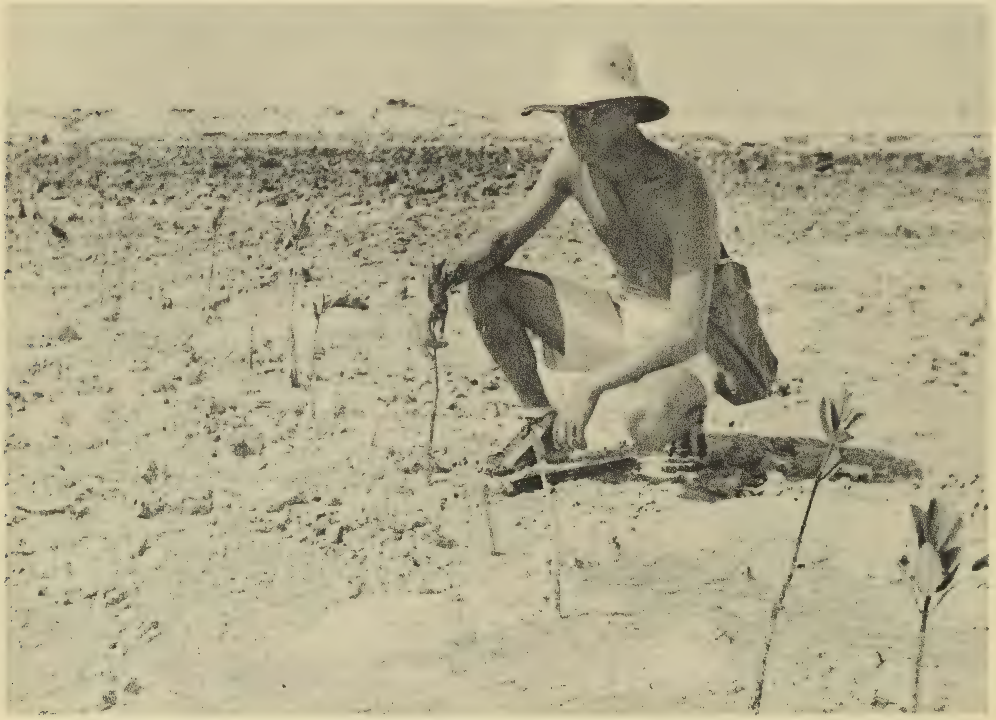


Fig. 2. Planting of *Rhizophora* seedlings on Long Key at the Tortugas. After one year 68 of 100 planted survived. The top growth is about 1 foot. Photograph taken at low tide; water covers the plants at high tide.

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